THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

Kinship and sociability in eastern grey kangaroos

Wendy Jane King

BSc (Hon), MSc

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2015

School of Biological Sciences

Frontispiece: A family group of eastern grey kangaroos at the Wilsons Promontory National Park in February, 2010, consisting of (left to right) adult female #6 (carrying a 3-month-old pouch young daughter that became #223), adopted young-at-foot daughter #116 (still suckling at approximately 15 months) and sub-adult daughter #7 of approximately 26 months. Photo by Wendy J. King.

Abstract

Animal grouping patterns are generally shaped by ecological constraints such as resource distribution or predation risk. Population density often increases competition, so that life-history consequences of association patterns should be particularly evident at increased densities. This thesis seeks to quantify how kinship affects association patterns in a species with unstable social bonds at high density. I also aimed to measure individual variation in sociability and evaluate its fitness consequences. Eastern grey kangaroos (*Macropus giganteus*) are gregarious herbivores, with a fission-fusion social system. I thus investigated how kinship and sex-age class affected behavioural and spatial associations in a high-density population of kangaroos at Wilsons Promontory National Park, Victoria.

In Chapter 2, I examined relationships between individuals of different sex-age classes using half-weight indices (HWIs, which measure pairwise association strengths) and lagged association rates, and calculated six individual-based network measures. I found little social structure in the population. Mean HWIs were similar within and between different sex-age combinations (mean HWIs = 0.01 to 0.02). Individuals of all sex-age classes had preferred and avoided associates overall. Adult females showed a weak relationship ($r = 0.05$) between relatedness and HWIs, but this effect disappeared when controlling for geographic distance or spatial overlap between dyads. Lagged association rates decreased exponentially for adult males with adult females accompanied by young-at-foot in the breeding season. Most other lagged association rates decreased slightly in a linear fashion and few were constant. Although females without young-at-foot appeared to be more social than females with young-at foot, all associations among individual non-juvenile kangaroos were weak (mean HWI = 0.01). Sample size had strong positive effects on mean HWI and all six network measures ($r = 0.40$ to 0.66), which is more likely to occur in large populations where association strengths are generally low and individuals have many weak associations.

I then combined observational data with genetic information to investigate sex-biased dispersal in Chapter 3. I found weak fine-scale genetic structure among adult females but none among adult males. Mean relatedness among adults was low ($r = –0.01$ to $–0.03$). Immature males moved away from their mothers at a younger age than did immature females. Although median distances of detected dispersals were 2–2.5 km for both sexes, only 6% of sub-adult females dispersed compared to 34% of sub-adult males. Adult females had overlapping ranges far wider than the maximum

extent of spatial genetic structure found. My results suggest that negative density dependence shapes spatial genetic structure.

In Chapter 4, I examined development of the mother-offspring relationship after permanent emergence from the pouch. Mothers associated more closely with their juvenile daughters than with their sons, but neither sex associated closely with their mothers as adults. There were few differences in the behaviour of juvenile sons and daughters towards mothers, although daughters foraged slightly closer than sons to their mothers, and sons were sometimes weaned earlier than daughters if the mother had a subsequent pouch young. Mothers never intervened to defend their young-at-foot from aggressive individuals and it was the offspring that maintained spatial proximity to their mothers. Because kangaroo mothers have few interactions with their juvenile offspring other than nursing, it is unlikely that a close bond develops between mothers and young.

Chapter 5 reports on eight adoptions that were detected through observations of marked mothers and young, and three more using microsatellite DNA. Four adoptions involved reciprocal switches and three were by mothers whose own pouch young disappeared. Adoptive mothers were not closely related to each other or to adoptees. Adoptive mothers and young associated as closely as did biological pairs. Adoptions tended to be more likely in high-density years and were probably caused by misdirected care, suggesting poorly developed mother-offspring recognition.

Finally, in Chapter 6 I aimed to determine whether the strength of early social relationships correlated with offspring survival, growth and reproduction. Association indices with the mother and time spent alone with the mother were not correlated with juvenile survival, body condition as 2-year-olds or reproduction as 3-year-olds. Sons and daughters that spent a large proportion of time with their mothers at 18–21 months, however, were subsequently 6% larger and 18% heavier as 2-year-olds than those that did not associate with their mothers. Mother-offspring associations before weaning thus had a beneficial effect on growth, which likely reflects maternal care.

I have shown in this thesis that weak but non-random associations occurred among individuals in this high-density population. Weak associations among adult females resulted from philopatry but were not based on kinship because density decreased mean relatedness among neighbouring females. Strong associations occurred between adult females and their young-at-foot and the strength of mother-offspring associations had a positive effect on juvenile growth. The benefits of these associations, however, were likely a direct result of greater maternal care rather than reduced predation or increased access to resources.

Declaration by author

This thesis *is composed of my original work, and contains* no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted *to qualify for the award of any* other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Peer-reviewed papers:

King, WJ, Garant, G, Festa-Bianchet, M (2015) Mother-offspring distances reflect sex differences in fine-scale genetic structure of eastern grey kangaroos. *Ecology and Evolution* 5:2084-2094.

King, WJ, Forsyth, DM, Coulson, G, Festa-Bianchet, M (2015) Adoption in eastern grey kangaroos: a consequence of misdirected care? *PLoS One* 10:e0125182.

King, WJ, Wilson, ME, Allen, T, Festa-Bianchet, M, Coulson, G (2011) A capture technique for free-ranging eastern grey kangaroos (*Macropus giganteus*) habituated to humans. *Australian Mammalogy* 33: 47-51

Publications included in this thesis

King, WJ, Garant, G, Festa-Bianchet, M (2015) Mother-offspring distances reflect sex differences in fine-scale genetic structure of eastern grey kangaroos. *Ecology and Evolution* 5:2084-2094– incorporated as Chapter 3.

Contributor	Statement of contribution
Wendy J. King (Candidate)	Designed study (70%) Performed fieldwork (80%) Analysed the data (100%) Wrote the paper (90%)
Dany Garant	Designed study (20%) Supervised laboratory analyses (100%) Wrote the paper (10%) Edited paper (50%)
Marco Festa-Bianchet	Designed study (10%) Performed fieldwork (20%) Edited paper (50%)

King, WJ, Forsyth, DM, Coulson, G, Festa-Bianchet, M (2015) Adoption in eastern grey kangaroos: a consequence of misdirected care? *PLoS One* 10:e0125182– incorporated as Chapter 5.

Contributor	Statement of contribution
Wendy J. King (Candidate)	Designed study (50%) Performed fieldwork (40%) Analysed the data (100%) Wrote the paper (100%)
David M. Forsyth	Designed study (10%) Performed fieldwork (10%) Edited paper (30%)
Graeme Coulson	Designed study (10%) Performed fieldwork (20%) Edited paper (30%)
Marco Festa-Bianchet	Designed study (30%) Performed fieldwork (30%) Edited paper (40%)

King, WJ, Wilson, ME, Allen, T, Festa-Bianchet, M, Coulson, G (2011) A capture technique for free-ranging eastern grey kangaroos (*Macropus giganteus*) habituated to humans. *Australian Mammalogy* 33: 47-51– incorporated as Appendix 1.

Contributor	Statement of contribution
Wendy J. King (Candidate)	Designed study (50%) Performed fieldwork (20%) Analysed the data (100%) Wrote the paper (100%)
Michelle E. Wilson	Performed fieldwork (20%) Edited paper (20%)
Teigan Allen	Performed fieldwork (10%) Edited paper (20%)

Marco Festa-Bianchet	Designed study (20%) Performed fieldwork (30%) Edited paper (30%)
Graeme Coulson	Designed study (30%) Performed fieldwork (20%) Edited paper (30%)

Contributions by others to the thesis

Genotyping of DNA samples for Chapters 2, 3, and 5 was performed by Jennifer Chambers, Hélène Pressault-Gauvin and Élise Rioux-Paquette. Marco Festa-Bianchet provided some observational data for Chapter 4. David Forsyth provided density estimates for Chapter 5. Simon Blomberg provided statistical advice for Chapters 4 and 6.

Statement of parts of the thesis submitted to qualify for the award of another degree

None.

Acknowledgements

First I would like to thank my supervisors for their persistence in this long endeavour. Anne Goldizen helped me see the big picture beyond the details and Graeme Coulson always had useful insights on macropod and mammalian biology. Michael Noad brought a cetacean aspect to discussions. Hugh Possingham and especially Diana Fisher were instrumental as critical readers. Thanks are also due to Dan Blumstein and Elissa Cameron for constructive criticism as external examiners. This thesis benefitted from discussions with Marc Belisle, Ian Beveridge, Alecia Carter, Mark Elgar, David Forsyth, Dany Garant, Emily Hynes, Jens Krause, David McDonald, Fanie Pelletier, Gabriel Ramos-Fernández, Greta Frankham, Denis Réale, Kathreen Ruckstuhl, Jennifer Seddon and Eric Vander Wal.

Funding was received as a living expenses scholarship from the Natural Sciences Research Council of Canada and a fee remission scholarship from UQ. Bishop's University gave me money for 'perfectionnement'. I had grants from the Holsworth Wildlife Research Endowment for field expenses. Various organisations funded trips to conferences: the Australasian Society for the Study of Animal Behaviour, the Australian Mammal Society, the International Society for Behavioral Ecology, and the School of Biological Sciences at UQ.

This research project would not have been possible without the input of many people in the field. Parks Victoria provided accommodation and logistic support. Rangers Dave Bone, Scott Griggs, Matt Hoskins, Dan Jones, Brett Mitchell, Dan Rogers and Jim Whelan were particularly helpful. I am eternally grateful to Nick Ryan for rescuing me when I exhausted my car battery at the airstrip one evening. Several biologists captured and marked the kangaroos that I was then able to identify as individuals: Graeme Coulson, Marco Festa-Bianchet, Uriel Gélin and Emily Hynes. Camille Le Gall-Payne and Ruth Glass helped monitor the population. Mike Johnstone from the Arthur Rylah Institute of the Department of Environment and Primary Industries made a caravan available for the 6 months when I was unable to access the Botany Laboratory accommodation due to flooding. Dave Forsyth provided density estimates. Uriel Gélin, Emily Hynes and Élise Rioux-Paquette kept me company.

The Zoology Department at the University of Melbourne was a second home from UQ while I was doing fieldwork in Victoria over 27 months. I greatly benefitted from discussions at the Conservation and Climate Change lab meetings and also at the Evolution and Behaviour meetings.

Kim Knowles kept my laptop running and helped with installation of computer software. Josie d'Alessandro maintained my sanity from time to time.

At UQ, members of the Behavioural Ecology Research Group provided friendship and collegiality. Vera Weisbecker granted me access to her lab when BERG was translocated to Long Pocket during the renovations. Simon Blomberg helped with statistical analyses. Gail Walters kept me on track.

The Université de Sherbrooke hosted me during the final writing stages of the thesis. Fanie Pelletier allowed me to use a personal computer for certain programs that would not run on my Macintosh. Jennifer Chambers, Hélène Pressault-Gauvin and Élise Rioux-Paquette performed the genotyping. Miriam Bass assisted with data entry. Camille Le Gall-Payne provided the photograph of a mother with an adopted pouch young.

Many housemates put up with my ranting and raving about student life over the years. Thanks to Claire Boulanger, Eddie Chung, Agnes Gelmini, Tamara Johnson, David Judson, Coralie Reich, Zoe Revie, James Sadler and Brooke Searle for listening and also to Nicole Barbee and Steve Swearer for letting me stay in their beautiful Melbourne home. Stephen Frankenberg, Kylie Gorringe, Genevieve Morris, Coralie Reich and Matt Symonds organised Friday afternoon get-togethers. Lisa Hodgkin, Tamara Johnson, Jess Roberts and Eunice Tan occasionally spiced up life with outings.

Finally, a special thanks to my family for letting me travel across the world to pursue my PhD adventure. My daughter Jennifer gave me unconditional support and my son Simon helped with captures and MATLAB. My husband Marco arranged to spend a sabbatical year in Australia and thus pulled me out of wombat holes and made sure I got home at night for the 16 months that we overlapped. Thanks for all the short- and long-distance support.

This thesis is dedicated to the memory of Don Thomas; researcher, mentor and friend. Here's hoping it's at least half as good as he would have expected.

Keywords

Kangaroo, marsupial, spatial autocorrelation, sociability, kinship, maternal care, adoption.

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 060201, Behavioural Ecology, 40%

ANZSRC code: 060801, Animal Behaviour, 40%

ANZSRC code: 060411, Population, Ecological and Evolutionary Genetics, 20%

Fields of Research (FoR) Classification

FoR code: 0602, Ecology, 40%

FoR code: 0608, Zoology, 40%

FoR code: 0604, Genetics, 20%

Table of Contents

ABSTRACT ..... **III**

DECLARATION BY AUTHOR ..... **V**

PUBLICATIONS DURING CANDIDATURE ..... **VI**

PUBLICATIONS INCLUDED IN THIS THESIS ..... **VI**

ACKNOWLEDGEMENTS ..... **X**

TABLE OF CONTENTS ..... **XIII**

LIST OF TABLES & FIGURES ..... **XVI**

TABLES XVI

FIGURES XIX

LIST OF ABBREVIATIONS USED IN THE THESIS ..... **XXIV**

CHAPTER 1: GENERAL INTRODUCTION **1**

1.1 GROUPING PATTERNS 1

1.1.1 Presence of Kin *3*

1.1.2 Sociability *4*

1.2 MACROPODIDS 5

1.3 STUDY SITE AND ANIMALS 8

1.4 OBJECTIVES AND THESIS STRUCTURE 9

1.5 ORIGINALITY OF RESEARCH 10

1.6 REFERENCES 11

CHAPTER 2: GROUPING PATTERNS SHOW SUBTLE SOCIAL STRUCTURE IN A SPECIES WITH STRONG FISSION-FUSION DYNAMICS **23**

2.1 ABSTRACT 23

2.2 INTRODUCTION 23

2.3 MATERIALS AND METHODS 25

2.3.1 Study Area and Animals *25*

2.3.2 Analyses *27*

2.3.3 Lagged Association Rates *29*

2.4 RESULTS 30

2.4.1 General Social Structure *30*

2.4.2 Effects of Sample Size on Individual Network Measures *32*
2.4.3 Temporal Patterns of Associations *32*
2.5 DISCUSSION 33
2.6 ACKNOWLEDGEMENTS 36
2.7 REFERENCES 36

CHAPTER 3: MOTHER-OFFSPRING DISTANCES REFLECT SEX DIFFERENCES IN FINE-SCALE GENETIC STRUCTURE OF EASTERN GREY KANGAROOS 68
3.1 ABSTRACT 68
3.2 INTRODUCTION 68
3.3 MATERIALS AND METHODS 70
3.4 RESULTS 74
3.4.1 Microsatellite Analyses *74*
3.4.2 Spatial Autocorrelation *74*
3.4.3 Observational Data *75*
3.5 DISCUSSION 76
3.6 ACKNOWLEDGEMENTS 77
3.7 REFERENCES 77

CHAPTER 4: SEX EFFECTS IN THE ONTOGENY OF MOTHER-OFFSPRING RELATIONSHIPS IN EASTERN GREY KANGAROOS 96
4.1 ABSTRACT 96
4.2 INTRODUCTION 96
4.3 METHODS 98
4.3.1 Study Site, Population and Captures *98*
4.3.2 Focal Sampling *99*
4.3.3 Instantaneous Sampling *101*
4.3.4 All Occurrences Sampling *101*
4.3.5 Statistical Analyses *102*
4.4 RESULTS 103
4.4.1 Birth Dates and General Behaviour of Mothers *103*
4.4.2 Sex Difference in Spatial Measures *104*
4.4.3 Sex Difference in Age Last Suckling and in the Pouch *104*
4.4.4 Sex Difference in Amounts of Affiliative and Aggressive Interactions with the Mother *105*
4.4.5 Sex Difference in Frequency of Distress Calls *105*
4.4.6 Sex Difference in Time Spent with the Mother *106*

4.5 DISCUSSION 106
4.6 ACKNOWLEDGEMENTS 109
4.7 REFERENCES 109

CHAPTER 5: ADOPTION IN EASTERN GREY KANGAROOS: A CONSEQUENCE OF MISDIRECTED CARE? 125

5.1 ABSTRACT 125
5.2 INTRODUCTION 125
5.3 MATERIALS AND METHODS 126
5.4 RESULTS 128
5.5 DISCUSSION 129
5.6 ACKNOWLEDGEMENTS 132
5.7 REFERENCES 132

CHAPTER 6: BENEFITS OF MOTHER-OFFSPRING ASSOCIATIONS IN EASTERN GREY KANGAROOS 143

6.1 ABSTRACT 143
6.2 INTRODUCTION 143
6.3 METHODS 145
6.4 RESULTS 148
6.5 DISCUSSION 149
6.6 ACKNOWLEDGEMENTS 151
6.7 REFERENCES 152

CHAPTER 7: GENERAL CONCLUSION 165

7.1 SUMMARY 165
7.2 FUTURE DIRECTIONS 167
7.3 REFERENCES 169

APPENDIX 1: A CAPTURE TECHNIQUE FOR FREE-RANGING EASTERN GREY KANGAROOS (*MACROPUS GIGANTEUS*) HABITUATED TO HUMANS 173

List of Tables & Figures

TABLES

CHAPTER 2

Table 1. Attributes of the social structure of marked eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. 42

Table 2. Mean HWIs for different combinations of 6 sex-age classes of 153 marked eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. 43

Table 3. Mean maximum HWIs for different combinations of 6 sex-age classes of 153 marked eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. 44

Table 4. Pearson correlation coefficients (*r*), *P*-values and equations for relationships between sample size and several measures of sociability for 153 eastern grey kangaroos seen a minimum of 10 times and 136 kangaroos seen a minimum of 40 times at Wilsons Promontory National Park, Australia, October 2010 to June 2011. 45

Table 5. Lagged association rate models fitted to 21 sex-age class combinations of 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. 46

CHAPTER 3

Table 1. Results of global autocorrelations between genetic and geographic distances for adult female and male eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2010–2011, 2011–2012 and both years combined. 83

Table 2. Results of autocorrelations between genetic and geographic distances for adult female eastern grey kangaroos in increasing distance classes at Wilsons Promontory National Park, Australia, 2010–2011 and 2011–2012. 84

Table 3. Results of autocorrelations between genetic and geographic distances for adult male eastern grey kangaroos in increasing distance classes at Wilsons Promontory National Park, Australia, 2010–2011 and 2011–2012. 85

Table 4. Median distance (m) between the centroids of 85 eastern grey kangaroo mothers and their offspring of different ages at Wilsons Promontory National Park, Australia, April 2010–June 2012. 86

Table 5. Final model of a general linear mixed model of distances between centroids (log-transformed) of 85 mothers and 134 offspring for eastern grey kangaroos at Wilsons Promontory National Park, Australia, April 2010–June 2012, $n = 404$. 87

Table 6. Mean range sizes and widths based on 95% and 50% kernels for eastern grey kangaroos of different sex-age classes at Wilsons Promontory National Park, Australia, October 2010–June 2011. 88

Supplementary Table S1. Microsatellite loci details for multiplex PCR amplification. 89

Supplementary Table S2. Four polymerase chain reaction recipes for the 9 microsatellite loci used in Table S1. 90

Supplementary Table S3. Size range, number of alleles, observed (H_o) and expected (H_e) heterozygosity and probability of deviation from Hardy-Weinberg equilibrium (*P* H-W) for 9 microsatellite loci in 176 adult eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2010–2012. 91

CHAPTER 4

Table 1. Parameters for fixed effects in the final linear mixed-effects model of proportion time vigilant for 50 eastern grey kangaroo mothers at Wilsons Promontory National Park, Australia ($N = 365$). 115

Table 2. Parameters for fixed effects in the final linear mixed-effects model of mean distance between 50 mothers and 55 offspring in eastern grey kangaroos at Wilsons Promontory National Park, Australia ($N = 365$). 116

Table 3. Parameters for fixed effects in the final generalised linear mixed-effects model using logistic regression of whether Hinde's proximity index was positive or negative for 50 mother-offspring pairs aged 10.0 to 21.4 months in eastern grey kangaroos at Wilsons Promontory National Park, Australia ($N = 312$). 117

Table 4. Parameters for fixed effects in the final generalised linear mixed-effects model using logistic regression of whether offspring were seen suckling or not for 112 mother-offspring pairs aged 10.0 to 21.4 months in eastern grey kangaroos at Wilsons Promontory National Park, Australia ($N = 201$). 118

Table 5. Mean number (± 1SE) of mother-offspring interactions and distress calls per day seen for eastern grey kangaroos aged 10–13 months (Period 1), 14–17 months

(Period 2) and 18–21 months (Period 3; *N*=107 juveniles) at Wilsons Promontory National Park, Australia. 119

Table 6. Mean number (± 1SE) of mother-offspring interactions and distress calls per day seen for male and female eastern grey kangaroos aged 10–13 months (Period 1), 14–17 months (Period 2) and 18–21 months (Period 3; *N*=107 juveniles) at Wilsons Promontory National Park, Australia. 120

Table 7. Final model of a linear mixed-effects analysis of HWIs between mothers and offspring for eastern grey kangaroos at Wilsons Promontory National Park, Australia, observed between April 2010 and June 2012, $N = 427$. 121

CHAPTER 5

Table 1. Characteristics of adopted pouch young (PY) eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2008–2013. 136

Table 2. Characteristics of adoptive female eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2008–2013. 137

Table 3. Parameter estimates for fixed effects retained in the generalised linear mixed-effects logistic regression model of whether or not 11 adult female eastern grey kangaroos adopted a pouch young at Wilsons Promontory National Park, Australia, 2009–2013 ($n = 43$). 138

Table 4. Parameter estimates for fixed effects retained in the generalised linear mixed-effects logistic regression model of offspring survival to 21 months for 11 adult female eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2009–2013 ($n = 43$). 139

Supplementary Table S1. Numbers of marked adult female kangaroos raising an offspring to large pouch stage (approximately 8 months of age) each year from 2008 to 2013 at Wilsons Promontory National Park, Australia. 140

CHAPTER 6

Table 1. Sample sizes of 129 juvenile eastern grey kangaroos observed in three different cohorts from April 2010 to June 2012 at Wilsons Promontory National Park, Australia. 155

Table 2. Final model of a linear mixed-effects analysis of proportion time offspring spent alone with their mothers while actively foraging for eastern grey kangaroos at

Wilsons Promontory National Park, Australia, observed between April 2010 and June 2012, *n* = 244. 156

Table 3. Final model of ANCOVA for size of the hind leg (in mm) of sub-adult eastern grey kangaroos first captured as large pouch young and then recaptured approximately 18 months later at Wilsons Promontory National Park, Australia, between August 2009 and March 2013, *n* = 47. 157

Table 4. Final model of ANCOVA for mass (in kg) of sub-adult eastern grey kangaroos first captured as large pouch young and then recaptured approximately 18 months later at Wilsons Promontory National Park, Australia, between August 2009 and March 2013, *n* = 44. 158

Table 5. Final model of ANCOVA for body condition of sub-adult eastern grey kangaroos first captured as large pouch young and then recaptured approximately 18 months later at Wilsons Promontory National Park, Australia, between August 2009 and March 2013, *n* = 55. 159

Table 6. Final model of generalised mixed-effects logistic regression for survival to 21 months of eastern grey kangaroos first captured as large pouch young at Wilsons Promontory National Park, Australia, between August 2009 and December 2011, *n* = 113. 160

FIGURES

CHAPTER 1

Figure 1. Steps leading to the formation of long-term bonds or temporary associations in animals. 20

Figure 2. Reproductive cycle of eastern grey kangaroos, showing a) overlapping gestations and lactations of normal cycling in blue and b) the possibility of mating while carrying a pouch young and then retaining a dormant blastocyst for several months in red. 21

Figure 3. Mean height (± 1SE) of palatable species for eastern grey kangaroos at Wilsons Promontory National Park, Australia, September 2010–August 2012, commencing in autumn (Month 3 = March). 22

CHAPTER 2

Figure 1. Aerial photo of the study area at Wilsons Promontory National Park, Australia, showing the 'main' area observed outlined in yellow and the entire area surveyed in red. 47

Figure 2. Distribution of nearest-neighbour distances for eastern grey kangaroos at Wilsons Promontory National Park, Australia, April 2010 to June 2012 (n = 21,110). 48

Figure 3. Principal component analysis of HWI values for 153 eastern grey kangaroos at Wilsons' Promontory National Park, Australia, October 2010 to June 2011. 49

Figure 4. Relationship between geographic distance and HWI for 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011 (n = 11,628). 50

Figure 5. Relationship between pairwise relatedness and HWI for 76 adult female eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011 (n = 2,850). 51

Figure 6. Mean proportion time spent alone (± 1SE) for 153 eastern grey kangaroos of different sex-age classes at Wilsons Promontory National Park, Australia, October 2010 to June 2011. 52

Figure 7. Relationship between sample size and mean HWI for different sex-age classes of 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. 53

Figure 8. Relationship between sample size and number of associates for 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. 54

Figure 9. Relationships between sample size and reach (closed circles and solid line), strength (open circles and dashed line) and affinity (crosses and dotted line) for 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia October 2010 to June 2011. 55

Figure 10. Relationships between sample size and eigenvector centrality (closed circles and solid line) and clustering coefficient (crosses and dotted line) for 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. 56

Supplementary Figure S1. Lagged association rates for AFY-AFY averaged over 7,000 associations. 57

Supplementary Figure S2. Lagged association rates for AFN-AFY averaged over 3,000 associations. 57

Supplementary Figure S3. Lagged association rates for AFN-AFN averaged over 2,000 associations. 58
Supplementary Figure S4. Lagged association rates for AM-AFY averaged over 2,000 associations. 58
Supplementary Figure S5. Lagged association rates for AM-AFN averaged over 1,000 associations. 59
Supplementary Figure S6. Lagged association rates for AM-AM averaged over 1,500 associations. 59
Supplementary Figure S7. Lagged association rates for SM-AFY averaged over 800 associations. 60
Supplementary Figure S8. Lagged association rates for SM-AFN averaged over 400 associations. 60
Supplementary Figure S9. Lagged association rates for SM-AM averaged over 500 associations. 61
Supplementary Figure S10. Lagged association rates for SM-SM averaged over 400 associations. 61
Supplementary Figure S11. Lagged association rates for SAF-AFY averaged over 300 associations. 62
Supplementary Figure S12. Lagged association rates for SAF-AFN averaged over 200 associations. 62
Supplementary Figure S13. Lagged association rates for SAF-AM averaged over 700 associations. 63
Supplementary Figure S14. Lagged association rates for SAF-SM averaged over 400 associations. 63
Supplementary Figure S15. Lagged association rates for SAF-SAF averaged over 1,200 associations. 64
Supplementary Figure S16. Lagged association rates for SAM-AFY averaged over 3,000 associations. 64
Supplementary Figure S17. Lagged association rates for SAM-AFN averaged over 2,000 associations. 65
Supplementary Figure S18. Lagged association rates for SAM-AM averaged over 1,000 associations. 65
Supplementary Figure S19. Lagged association rates for SAM-SM averaged over 700 associations. 66

Supplementary Figure S20. Lagged association rates for SAM-SAF averaged over 2,000 associations. 66
Supplementary Figure S21. Lagged association rates for SAM-SAM averaged over 3,000 associations. 67

CHAPTER 3

Figure 1. Correlograms for adult female eastern grey kangaroos at Wilsons Promontory National Park, Australia, in a) 2010–2011 and b) 2011–2012, comparing genetic distance to spatial distance in m, using 25-m distance classes. 92
Figure 2. Distance between centroids of eastern grey kangaroo mothers and their offspring for 4-month age periods starting at 10 months of age, at Wilsons Promontory National Park, Australia, April 2010–June 2012. 93
Figure 3. Map of 50 adult male (closed circle) and 106 adult female (open circle) centroids of marked eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2011–2012. 94
Figure 4. Pairwise relatedness coefficients *r* plotted against distance between centroids for adult female eastern grey kangaroos that had centroids within 25 m at Wilsons Promontory National Park, Australia, 2010–2011 (closed circles, n = 38) and 2011–2012 (crosses, *n* = 66). 95

CHAPTER 4

Figure 1. Distribution of 189 eastern grey kangaroo births at Wilsons Promontory National Park, 2008-2011 122
Figure 2. Rates of nursing (± 1SE) between adult female and juvenile eastern grey kangaroos of different sexes and ages at Wilsons Promontory National Park, Australia, between April 2010 and June 2012. 123
Figure 3. Half-weight indices (HWIs) between 138 eastern grey kangaroos aged 10–53 months and their mothers at Wilsons Promontory National Park, Australia. 124

CHAPTER 5

Figure 1. Female #81 with adopted young #531 in her pouch. 141
Figure 2. Annual percentage adoptions as a function of density of female eastern grey kangaroos with large pouch young (LPY) in winter at Wilsons Promontory National Park, Australia. 142

CHAPTER 6

Figure 1. Deaths or disappearances of juvenile eastern grey kangaroos (n = 80) in 3 cohorts of juveniles at Wilsons Promontory National Park, Australia, that were marked from August 2009 to December 2011 at approximately 8 months of age and followed to December 2012. 161

Figure 2. Mean proportion of time (± 1SE) sons and daughters spent alone with their eastern grey kangaroo mother while actively foraging at ages 10–13 months, 14–17 months and 18–21 months between April 2010 and June 2012 at Wilsons Promontory National Park, Australia. 162

Figure 3. Body condition (± 1SE) of female eastern grey kangaroos and their large pouch young captured at Wilsons Promontory National Park, Australia, 2009-2010 (n = 113 pairs). 163

Figure 4. Generalised linear logistic regression of body condition as a large pouch young on survival to 21 months for eastern grey kangaroos first captured at Wilsons Promontory National Park, Australia, between August 2009 and December 2011, n = 75. 164

List of Abbreviations Used in the Thesis

AFN	adult female without a young-at-foot
AFY	adult female with a young-at-foot
AM	large adult male
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BSA	bovine serum albumin
CV	coefficient of variation
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
GPS	global positioning system
H_e	expected heterozygosity
H_o	observed heterozygosity
HWI	half-weight index
ID	identity
K-W	Kruskal-Wallis test statistic
LPY	large pouch young
LAR	lagged association rate
PCA	principal component analysis
PCR	polymerase chain reaction
P H-W	probability of deviation from Hardy-Weinberg equilibrium
PY	pouch young
QAIC	quasi Akaike information criterion
REML	restricted maximum likelihood
SAF	sub-adult female
SAM	sub-adult male
SE	standard error
SM	small male
U	Mann-Whitney U test statistic
VIF	variance inflation factor

CHAPTER 1: General Introduction

1.1 GROUPING PATTERNS

Grouping patterns in animal societies are generally shaped by ecological constraints (Rubenstein and Wrangham 1986). Aggregations of animals occur in response to resource distribution, predator pressure or both (Figure 1) (Krebs and Davies 1997). Stable groups are those where individuals tend to associate over their lifetimes. At the other extreme, unstable groups may involve frequent turnover of individuals over space and time; individuals may join and leave these groups at will, with high fission-fusion dynamics. There is, however, a gradient between these two extremes of group types and extended associations between individuals can arise in the absence of ecological factors (Aureli et al. 2008). In some cases, social organisation can have a hierarchical structure of very stable subgroups, yet loose associations between subgroups (Whitehead 2008).

Animals in aggregations can have improved predator detection (the 'many-eyes' hypothesis (Lima 1995)), reduced individual risk of predation (due to the dilution effect (Cresswell 1994), confusion effect (Neill and Cullen 1974) or the 'selfish herd' hypothesis (Hamilton 1971)) and cooperative defense against predators (Heard 1992). In particular, the many-eyes hypothesis involves group-size effects, in that vigilance rates tend to decrease in large groups of foraging prey (Roberts 1996). Group-size effects on vigilance can be obscured in large groups, however, if conspecific threat increases with group size (Treves 2000).

Whether social animals form stable or unstable groups depends primarily on the nature of the ecological resources involved. Where those resources are defensible, for example when food, nest sites or mates occur in clumps, animals often associate in stable social groups characterised by territoriality and/or dominance hierarchies (Clutton-Brock 1989; Wolff 1993). Intra-group competition is then generally high and long-term bonds between individuals can result (Silk 2007). In contrast, where resources are non-defensible, such as when food is evenly distributed and predictable, the grouping pattern is more likely to be unstable, which is generally characterised by temporary associations and low intra-group competition (Gowans et al. 2008). Many exceptions to these general patterns exist, however, as grouping patterns are affected by multiple variables, the relative importance of which can vary over space and time (Clutton-Brock 1989). In particular, in some unstable groups, long-term bonds and alliances may develop as a result of mother-offspring associations or other social factors (Schultz et al. 2011).

In vertebrates, the resources that drive grouping patterns often differ for the sexes since the dispersion of females usually matches food resources, while males are more often limited by mating

opportunities (Emlen and Oring 1977). Females in stable social groups can sometimes thus cooperate to monopolise food and/or breeding sites. In contrast, male cooperation is more likely to involve monopolisation of access to females. Males and females may thus display different grouping patterns within the same population. Alliances between individuals should only occur in groups in which individuals associate over the long term and reciprocate helping behaviour (Trivers 1971).

In addition to the ecological factors of resource distribution and predator pressure, social factors such as infanticide risk may drive grouping patterns (Kappeler 1997; Sterck et al. 1997). Females may form alliances with males to avert infanticide by strange males (Van Schaik and Kappeler 1997; Ebensperger 1998). Coalitions among related females appears to have played an important role in the evolution of primate sociality (Schultz et al. 2011). Social bonds, including alliances and coalitions, occur when individuals spend time in close proximity and interact in an amicable or cooperative manner (Massen et al. 2010). Reinforcement of bonds occurs through repetitive interactions such as social grooming and food-sharing (Silk et al. 2010a) or greetings (Smith et al. 2011), however degree of bondedness can be difficult to measure (Dunbar and Schultz 2010).

The costs and benefits to grouping have been of central interest to behavioural ecologists for decades (Alexander 1974). In stable social groups, benefits can include increased access to resources, reduced risk of predation, and reduced male harassment and infanticide (Silk 2007). Costs may include increased competition for resources, parasite transmission and risk of detection by predators (Davies et al. 2012). Because of relatively weak intra-group competition, interactions between individuals in unstable societies tend to be subtle so that the costs of competition may be difficult to quantify, but parasite transmission, at least, should increase with strength of association (VanderWaal et al. 2014). Benefits to associates in societies showing high fission-fusion dynamics may include reduced risk of predation and increased access to resources gained through information transfer (Kerth 2008). In both stable and unstable societies, benefits should outweigh costs for the grouping behaviour to evolve (Krebs and Davies 1997).

Competition for resources is often density-dependent (Rosenzweig 1981), which can affect spacing patterns and reproductive success (Van Schaik 1983). Increases in density generally lead to increases in dispersal tendencies in birds and mammals (Mattysen 2005). As density increases in small territorial mammals, so does territorial defense and wounding (Wolff 1993). Life-history consequences of association patterns in unstable groups should also be particularly evident at increased densities.

Recent research has underlined the importance of individual differences in driving processes critical to population dynamics and evolutionary ecology (Saccheri and Hanski 2006). Individuals

within populations can differ in terms of a multitude of traits, such as age, sex, genetic make-up, parasite load and experience of local conditions. The next two sections deal with two individual traits: presence of kin and sociability.

1.1.1 *Presence of Kin*

The availability of close kin is an important source of individual variation because relatives can affect the ability of females to access resources, avoid predation and care for their offspring (Clutton-Brock 2002). When animals are philopatric, kin are likely to settle near each other and associate as adults (Michener 1983). Kin may thus form clusters within a community and behave cooperatively (Hamilton 1964), which can lead to kin selection (Maynard-Smith 1964). Even low levels of mean pairwise relatedness (such as coefficients of 0.05) may be important predictors of kin-directed behaviour in populations in which cooperation takes the form of increased tolerance (Wolf and Trillmich 2008). Individuals in many animal societies can thus choose to associate more often with kin than non-kin, although the costs and benefits of such kin-preferential behaviour may be explained through direct rather than indirect fitness (Griffin and West 2002).

Because most mammalian females are philopatric (Dobson 1982), neighbours are likely to be related and kin-differential behaviour may take the form of tolerance rather than cooperation. Animals with individual territories that are fixed in space become familiar with their neighbours and tend to treat them preferentially with reduced aggression. This situation occurs in many vertebrates and is known as the 'dear enemy' effect (Ydenberg et al. 1988). Examples include females in a number of vole species (*Microtus sp.* and *Clethrionomy sp.*) (Ostfeld 1990), Columbian ground squirrels (*Urocitellus columbianus*) (King 1989) and Galapagos sea lions (*Zalophus wollebaeki*) when hauled out on land (Wolf and Trillmich 2008). Animals can also exist in group territories that are tightly fixed in space. Female yellow-bellied marmots (*Marmota flaviventris*) and Belding's ground squirrels (*Urocitellus beldingi*) are philopatric and small groups of related females cooperatively defend territories (Sherman 1980; Armitage 1991).

Strong dominance hierarchies develop among females, with kin relationships playing an important role, in larger groups that defend group territories *e.g.,* lions (*Panthera leo*) (Packer et al. 1991) and many primates (such as macaques (*Macaca sp*.), baboons (*Papio sp*.) and vervets (*Cercopithicus aethiops*)) (Silk 2002). Where both sexes tend to disperse, large groups include unrelated individuals that cooperate to defend territories, and again dominance hierarchies develop from strong intra-group competition, as in meerkats (*Suricata suricatta*) (Clutton-Brock and Lukas 2012). Dominance hierarchies without territoriality occur in large groups of ungulates such as bighorn sheep (*Ovis canadensis*) (Festa-Bianchet 1991), mountain goats (*Oreamnos americanus*)

(Festa-Bianchet and Côté 2008) and feral horses (*Equus caballus*) (Clutton-Brock et al. 1976; Cameron et al. 2009), with kinship playing a minor role in the hierarchies among adult females, either due to dispersal or demographic patterns. Groups of related females form loose matrilines, however, in red deer (*Cervus elaphus*) (Clutton-Brock et al. 1982) and white-tailed deer (*Odocoileus virginianus*) (Aycrigg and Porter 1997), with males forming dominance hierarchies and at times forming bachelor groups outside the rut.

In societies showing high fission-fusion dynamics, animals often join and leave groups, potentially limiting opportunities for individuals to form strong bonds or dominance hierarchies. Among species, there is a gradient in the level of preferred associations between individuals, which vary in both duration and strength. Examples involving weak female associations are found in greater horseshoe bats (*Rhinolophus ferrumequinum*) (Rossiter et al. 2002), chimpanzees (*Pan troglodytes*), spider monkeys (*Ateles geoffroyi*) (Symington 1990), giraffes (*Giraffa camelopardalis*) (Carter et al. 2013), Grevy's zebra (*Equus grevyi*), onagers (*Equus hemionus khur*) (Sundaresan et al. 2007) and bottlenose dolphins (*Tursiops aduncus*) (Frère et al. 2010b). Kinship appears to play an important role in those female relationships in greater horseshoe bats and bottlenose dolphins. Related male chimpanzees and bottlenose dolphins form alliances to monopolise access to breeding females (Wrangham 1986; Connor et al. 1992; Morin et al. 1994). More complex female associations involving dominance hierarchies occur in species such as spotted hyaenas (*Crocuta crocuta*), where individuals often travel alone but may join or leave more cohesive, matrilineally related subgroups than in the examples above (Smith et al. 2008). In other cases, individuals in subgroups remain together for the long-term but subgroups can fuse and break apart from each other, forming a multi-tiered society. This occurs in African elephants (*Loxodonta africana*) (Wittemyer et al. 2005), killer whales (*Orcinus orca*) (Whitehead and Baird 2000) and sperm whales (*Physeter macrocephalus*) (Whitehead et al. 1991). The bonded individuals within stable subgroups tend to be females that are matrilineally related, although both sexes can be philopatric in killer whales, leading to long-term bonds between males and females (Whitehead and Baird 2000).

1.1.2 *Sociability*

Another individual characteristic that has recently received considerable attention is sociability, which refers to the tendency of animals to interact with or spend time in close proximity to conspecifics (Kohn et al. 2013). Different individuals tend to show consistent behavioural traits over time and in different situations; these traits are often measured along five correlated continua of shyness/boldness, exploration/avoidance, activity, aggressiveness and sociability (Réale et al.

2007). Correlations both within and between individuals can be assessed for other behavioural traits (Dingemanse and Dochtermann 2013), including maternal behaviour (Koski 2014). Temperament has been extensively investigated in non-human primates, although mainly in captivity. The most commonly studied species are chimpanzees and rhesus macaques (*Macaca mulatta*), with sociability being one of the most frequently assessed and reliable traits (Freeman and Gosling 2010). Sociability can be evaluated through observations of an animal's propensity to interact with conspecifics or spend time alone (Freeman and Gosling 2010).

Assortative interactions between members of a population can result in animals associating with conspecifics of similar temperament (Croft et al. 2008). This means, for example, that sociable animals tend to associate with other sociable animals and shy animals tend to associate with shy conspecifics (Krause et al. 2000). Although several field studies have shown repeatability in individual temperament (Bell et al. 2009), it is important to evaluate the fitness consequences of variation in temperament in natural populations (Archard and Braithwaite 2010). There is now a growing number of studies correlating sociability with survival and/or reproduction in the wild (Cote et al. 2008; Cameron et al. 2009; Silk et al. 2009; Frère et al. 2010a; Silk et al. 2010b; Wey and Blumstein 2012; Kohn et al. 2013; McCowan et al. in press).

1.2 MACROPODIDS

Macropodids are medium-sized to large marsupial herbivores that can be viewed as the ecological equivalent of ungulates, particularly deer (Jarman 1983). Social structure and kin interactions among individually recognisable animals has been investigated in only a few species of this taxon. Reduced aggression occurs between related female tammar wallabies, *Macropus eugenii*, in captivity, however presence of close kin did not reduce vigilance levels (Blumstein et al. 2002). Resource competition between females and their sub-adult offspring is believed to reduce reproductive success in gregarious red-necked wallabies, *Macropus rufogriseus* (Johnson 1986). Females in at least one species (brush-tailed rock-wallabies, *Petrogale penicillata*) are highly philopatric (Hazlitt et al. 2004; Hazlitt et al. 2010). Most large macropods live in fission-fusion groups, with overlapping generations (Jarman 1991). Mothers accompanied by young-at-foot often tend to isolate themselves from other individuals in *Macropus giganteus* (Kirkpatrick 1966; Southwell 1984b; Jaremovic and Croft 1991; Jarman 1994), *M. fuliginosus* (Johnson 1983; Coulson 1993), *M. rufus* (Croft 1981a; Johnson 1983), *M. robustus* (Croft 1981b; Taylor 1983) and *M. antilopinus* (Croft 1982).

Eastern grey kangaroos (*Macropus giganteus*) are among the most gregarious of macropodids (Jarman 1991), with a fission-fusion social system (Southwell 1984b). There is a

prolonged period of maternal care (see Figure 2) and lactation lasts for approximately 18 months (Poole 1975). About half-way through lactation, the offspring permanently leaves the pouch and the mother can mate and give birth to a second offspring, however embryonic diapause may also occur (Poole and Catling 1974). The transition before permanent emergence from the pouch is called the 'in-and-out' period of pouch young development and lasts about 35 days (Poole and Catling 1974). Two siblings of different ages can be simultaneously nursed for approximately 6 months, with one offspring inside the pouch and the other associating with the mother as a young-at-foot (Stuart-Dick 1987). The mother-offspring bond that occurs during lactation could persist into adulthood and if female kin form close associations as adults, matrilines could develop. Preliminary analyses of kangaroo populations, however, reveal weak genetic structuring with mean levels of pairwise relatedness of 0.04, although there is evidence for male-biased dispersal (Zenger et al. 2003). Availability of close female kin in any population will depend on demographics (King and Murie 1985).

Kangaroos appear to form groups to reduce predation risk because individuals' vigilance levels usually decrease with increasing group size (Jarman 1987; Favreau et al. 2010). Potential costs to grouping include increased detectability by predators and increased feeding interference (Jarman and Coulson 1989). Individual differences in association indices (Carter et al. 2009a; Best et al. 2014) and vigilance levels (Carter et al. 2009b; Favreau et al. 2014) occur among females, which could both be explained by genetic relatedness within groups and/or sociability.

Studies of different sex-age grouping patterns have either found persistent, repeated association of individuals of various ages within the same group (Kaufmann 1975) or that group membership was random (Caughley 1964; Kirkpatrick 1966). Most studies report a modal group size of 2 in open habitats (Caughley 1964; Kaufmann 1974; Taylor 1982; Southwell 1984a; Stuart-Dick 1987). Jarman (1994) summarised observations of associations among approximately 100 individuals at low density (0.5 individuals/ha) at Wallaby Creek, NSW, over 4 years. He found that the different sex/age classes associated to varying extents. Small and intermediate-sized males appeared to frequently associate with each other. Large adult males tended to be solitary, reflecting the roving nature of their reproductive strategy. Adult females formed close units with their young-at-foot, a stage that lasts approximately 8 months, and avoided other females with young-at-foot. This avoidance was explained by the need to strengthen the mother-offspring bond at a time when the young must learn to identify and follow the mother (Jarman and Southwell 1986; Stuart-Dick 1987). The other classes of adult females, sub-adults and small adult males all showed positive associations with their peers (Jarman 1994), presumably because similar-sized animals have equivalent energetic requirements and hence can coordinate feeding and resting activities (Jarman and Coulson 1989). Persistent associations of individual females of similar reproductive status did

occur, which was believed to result from matrilineal relatedness (Stuart-Dick 1987). Adult females appeared to benefit from the presence of mothers and sisters by commencing breeding earlier and initially weaning more offspring than females without close kin (Jarman 1994).

More recently, Carter et al. (2009a) studied a population of 38 individually recognisable female kangaroos at moderately high density (approximately 2.0 individuals/ha) at Elanda Point, Queensland, over 9 months. Female kangaroos were found to have a non-random social structure that was not explained by the extent of home-range overlap between pairs of animals (Carter et al. 2009a). On average, females spent about 35% of their time with their closest female associate (Carter 2007). Kin relationships were unknown in this population, however, and sub-adults were not distinguished from adults.

Best et al. (2013) studied a large population of individually recognisable kangaroos for 2 years at moderately high density (approximately 2.0 individuals/ha) at Sundown National Park, Queensland, and confirmed that females formed preferential associations. Female kangaroos belonged to distinct communities and spent about 20% of their time with their closest female associate, on average (Best 2013). In addition, genetic information showed that bi-parental relatedness accounted for some female associations after controlling for spatial overlap (Best et al. 2014). Sub-adults were not distinguished from adults, however, and so the apparent effect of kinship may have been due to the presence of mother-immature daughter pairs.

Preliminary studies of kangaroo temperament at Sundown reveal consistent individual differences in personality and a weak negative correlation between boldness and foraging group size (Best et al. 2015). Boldness, however, did not correlate with other network metrics of personality, such as strength and affinity, or with association indices (Best et al. 2015). As yet, the common tendency found in large macropods for adult females to isolate themselves when accompanied by a young-at-foot has not been utilised as a measure of sociability in kangaroos. This measure cannot be used to assess reproductive success across all females, however, because reproductive status appears to dictate sociability (Croft 1981a).

The suggestion by Stuart-Dick (1987) and Jarman (1994) that adult females associate closely with kin in eastern grey kangaroos has not been tested in a framework excluding sub-adults. In addition, it is unknown whether kin associations and sociability affect reproductive success. If adult females behave like their cervid ecological equivalents, they should form loose matrilines, however deer do not associate in groups with high fission-fusion dynamics. Kangaroo society may thus instead resemble that of delphinids where related females form temporary associations.

1.3 STUDY SITE AND ANIMALS

I studied eastern grey kangaroos in southern Victoria, at an isolated 110-ha patch of grassy habitat around an emergency landing strip in Wilsons Promontory National Park (38° 57' S, 146° 17' E). In the early 1900s, an area of approximately 75 ha of acid sand was cleared and leveled to construct an airstrip for military purposes. Surrounding areas were used to run cattle but became parkland in 1969. The site now consists primarily of coast blown grass (*Lachnagrostis billardieri*), couch grass (*Bromus diandrus*) and salt-tolerant herbs growing amongst clumps of knobby club-rush (a sedge; *Ficinia nodosa*). There are areas with Austral bracken (*Pteridium esculentum*) and occasional trees and bushes such as coast tea-tree (*Leptospermum laevigatum*), golden wattle (*Acacia longifolia*) and coast banksia (*Banksia integrifolia*) (Davis et al. 2008). Other mammalian herbivores at the site include common wombats (*Vombatus ursinus*), swamp wallabies (*Wallabia bicolor*), hog deer (*Axis porcinus*) and European rabbits (*Oryctolagus cuniculus*). There are no wild dogs but predators include red foxes (*Vulpes vulpes*) and wedge-tailed eagles (*Aquila audax*). Rabbits are controlled by poison baiting and foxes are occasionally trapped. Park staff mechanically slash the sedges on the arms of the airstrip (an area of approximately 6 ha) twice a year; the airstrip was only used occasionally for fighting wildfires or emergency evacuations during my study. Mean daily maximum temperatures ranged between 14°C (July) and 25°C (January), while mean daily minimum temperatures were 6°C (July) to 13°C (February) (Australian Bureau of Meterology station #085099 at 38° 38' S, 145° 49' E). Mean monthly rainfall ranged from 29 mm (January) to 98 mm (August) and was usually above 70 mm from autumn through mid-spring.

Using a system of 54 quadrats (50 X 50 cm) spaced evenly (every 50 m) over the 'main' part (Chapter 2) of the study area, I measured the height (to the nearest 0.1 cm) of palatable herbaceous species at 5 points within each quadrat on a monthly basis between September 2010 and August 2012. Mean height of forage was low, varying from 1.7 to 3.1 cm throughout the year, being lowest in late winter (July and August; ANOVA, $F_{11,1229} = 5.78$, $P = 0.001$; Figure 3).

The population of eastern grey kangaroos at the airstrip originated from nine individuals that were held in captivity from 1910 and then accidentally released during a fire at Darby River, 3 km south of the airstrip, around 1938 (Whelan 2008). Kangaroos now occur throughout the park but are concentrated on the airstrip and surrounding meadows. Densities of kangaroos varied between 3 and 7 individuals/ha between August 2008 and July 2014 and peaked in the summer/autumn of 2011 (DF Forsyth, personal communication). Absolute numbers of kangaroos at the study site have thus been in the range of 300–750.

Kangaroos were marked for individual identification with eartags and collars (King et al. 2011). Ear tissue samples allowed genotyping using microsatellite markers (Chapter 3). Nearly all individuals were captured once a year to obtain measurements on size and mass, and to check on

reproduction of females (Chapter 6). I conducted intensive behavioural observations between April 2010 and June 2012 (Chapter 4), however my colleagues and I have monitored approximately 250 individuals in the population each year from 2010 to present.

1.4 OBJECTIVES AND THESIS STRUCTURE

The overall objective of my research was to quantify how kinship and other variables affected association patterns in a population of a species with fission-fusion grouping patterns at high density. Because mother-daughter relationships form the basis of many mammalian social systems, I wished to examine associations involving mother-offspring kinship pairs in detail and investigate maternal behaviour. I also aimed to measure individual variation in sociability and evaluate its fitness consequences. I thus studied different sex-age classes of eastern grey kangaroos at Wilsons Promontory National Park, Victoria, over 27 months. This thesis is written as manuscripts for publication in peer-reviewed journals, with Chapters 2 through 6 formatted in the style of the target journal. Consequently, there is some overlap in the presentation of the methods. Each chapter has its own References section. I submitted Chapter 3 for publication before Chapter 2, which explains some references to a later chapter in Chapter 2. The specific objectives of this thesis are as follows:

Chapter 2: Evaluate associations among individuals of different sex-age classes, as measured by shared foraging group membership, and describe kangaroo social structure. I used association indices and lagged association rates to determine association patterns. I also investigated the correlation between sample size and individual network measures.

Chapter 3: Determine the extent of philopatry of females and dispersal tendencies of males using both genetic information and observational data. I used spatial genetic autocorrelation, mother-offspring distances and opportunistic observations of emigrants.

Chapter 4: Assess the development of mother-offspring relationships and maternal behaviour after permanent emergence from the pouch, with a particular emphasis on potential sex differences. I used 1) association indices, 2) focal observations of mother-offspring distances, approaches/retreats, following and vigilance behaviours and 3) *ad lib* observations of interactions, nursing and distress calls.

Chapter 5: Describe adoptions of pouch young and relate them to hypotheses regarding kin selection, reciprocal altruism, improved parental experience and misdirected care. I used microsatellite DNA analyses and behavioural observations of mother-offspring pairs. Data on adoptions spanned 6 years.

Chapter 6: Determine whether the strength of early social relationships correlated with offspring survival, growth and reproduction. I compared association indices and proportion of time mothers spent alone with offspring to 1) offspring survival, 2) size and mass of 2-year-olds and 3) reproduction of 3-year-old daughters.

The final chapter discusses the influence of mother-offspring relationships and sociability in fission-fusion species and reflects on the suitability of kangaroos for this type of research. I have included a methodological publication as Appendix 1.

1.5 ORIGINALITY OF RESEARCH

My research into kinship and sociability of eastern grey kangaroos is based on a rigorous capture and marking program that started in 2008 and has now monitored over 800 individuals in a high-density population. No previous study on the social system of free-ranging macropods involved the marking of pouch young. Because we captured and marked nearly all pouch young of marked females each year, I was able to determine cohorts of offspring and easily recognise mothers and their offspring from a distance. I used measurements of pouch young size to determine birthdates (Poole et al. 1982). Accurately aging pouch young enabled me to classify young into different age groups, follow mother-young associations from permanent pouch emergence to adulthood and quantify social relationships among several different known sex-age classes. Capturing, marking and collection of tissue samples for DNA analyses from individuals also allowed direct measurement of body size and condition, genetic confirmation of mother-young associations, and identification of adoptions, carcasses and dispersing individuals. These are all novel aspects of my research.

The kangaroo study at Wallaby Creek (Jarman and Southwell 1986; Jarman 1994) evaluated associations among 8 different sex-age classes but combined the sexes for young-at-foot and sub-adults. Animals were not captured but recognised from distinguishing features. Ages of juveniles were estimated from their appearance. The researchers did not have access to modern molecular techniques to determine bi-parental relatedness using genotypes. Associations were not evaluated in terms of individual association indices or lagged association rates but by the sex-age class and

orientation of nearest neighbours and by the occurrence of different sex-age classes in different group types (*e.g.* alone). Maternal behaviour was studied (Stuart-Dick 1987) using focal observations of maternal vigilance, mother-offspring distances, directional movements of both mother and offspring while foraging, and interaction rates such as contacts, allogrooming, play and nursing, however sample sizes were small (7 daughters and 7 sons).

The Elanda Point and Sundown populations (Carter et al 2009a; Best et al. 2013) were only studied for up to two years and so the ages of sub-adults were unknown. Consequently, the analyses of associations among females using association indices (accounting for spatial overlap), lagged association rates and network measures combined sub-adult with adults. Associations among other sex-age classes were not investigated. Animals were not captured but recognised from distinguishing features. Genotypes at Elanda Point were unknown. Genotypes at Sundown were obtained from faecal samples, which can be difficult to amplify, so not all individuals were genotyped (Best et al. 2013). Fine-scale genetic structure was investigated at Sundown but only for females, pooling adults and sub-adults, and only at one distance class. Body condition was estimated from appearance, using an index that has not been validated for kangaroos. Sociability was assessed as mean group size and mean association indices.

My study of eastern grey kangaroos at high density at Wilsons Promontory thus builds upon but differs from those conducted at Wallaby Creek (low density) or Elanda Point and Sundown (intermediate density). No previous research has calculated lagged association rates for all sex-age classes, investigated the effect of sample size on individual network measures, examined fine-scale genetic structure in adults of both sexes, obtained the data necessary to document adoption of pouch young in the wild or evaluated the fitness consequences of early sociability. In conclusion, this is the first comprehensive study of the social system of a marsupial showing high fission-fusion dynamics with known genotypes, accurate ages of juveniles and sub-adults, robust sample sizes, and repeated individual morphometric measurements.

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Figure 1. Steps leading to the formation of long-term bonds or temporary associations in animals. The dotted line indicates that the temporal scale of associations may vary according to the scale of fission-fusion dynamics.

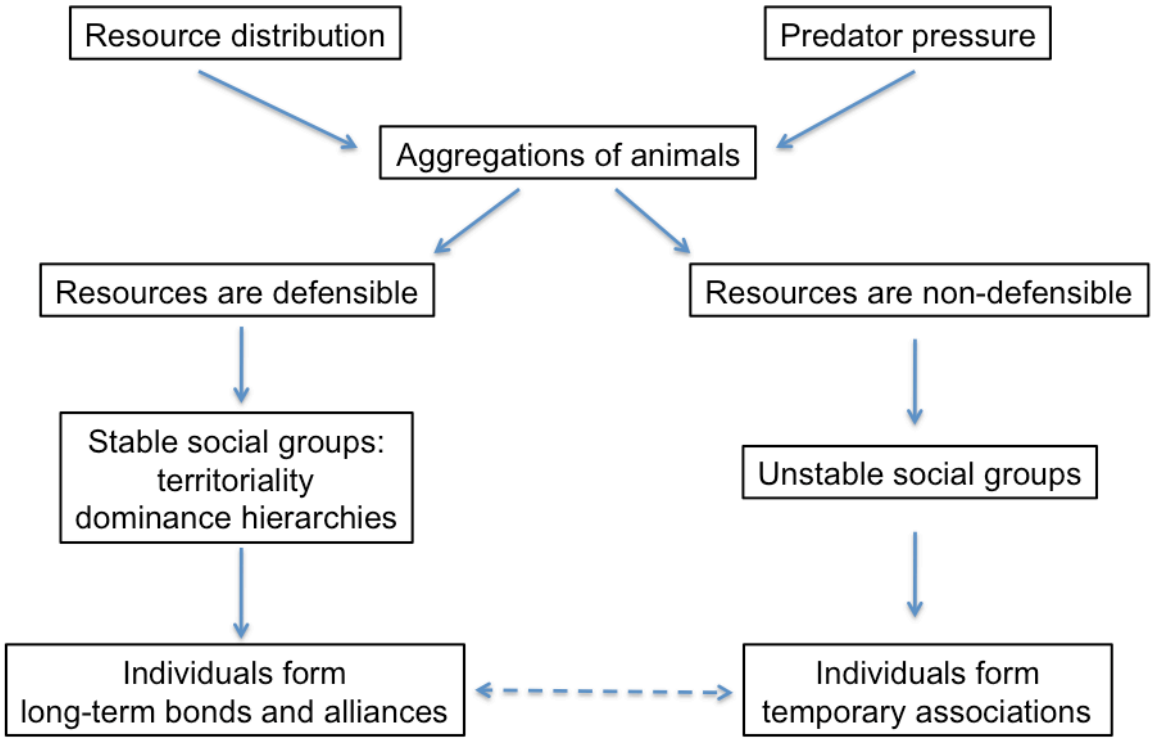
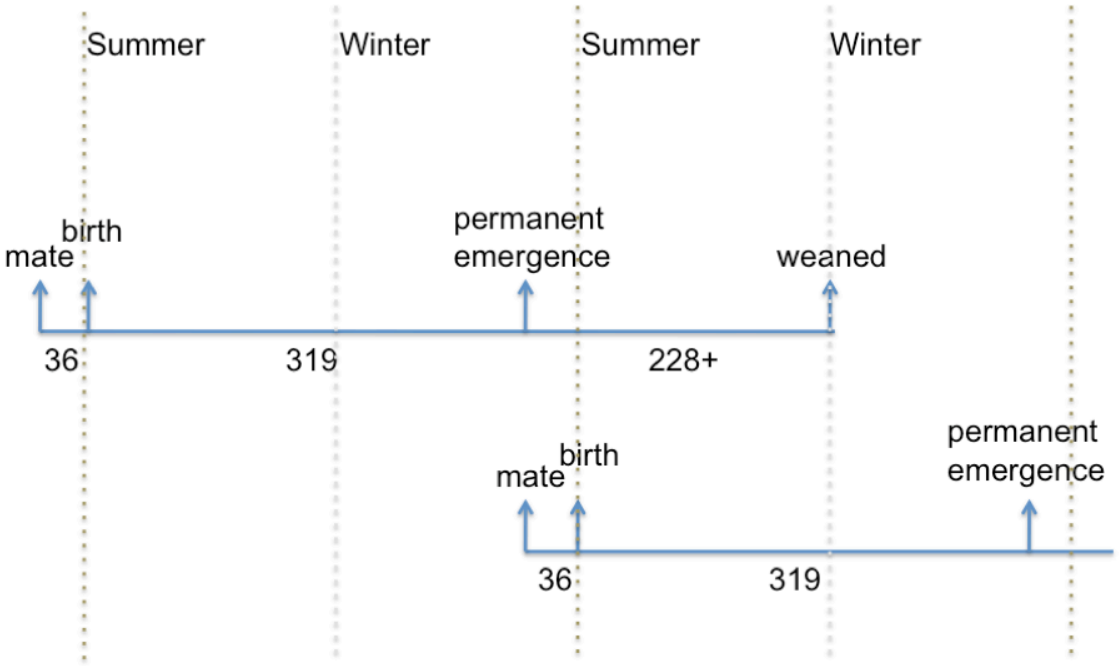


Figure 2. Reproductive cycle of eastern grey kangaroos, showing a) overlapping gestations and lactations of normal cycling in blue and b) the possibility of mating while carrying a pouch young and then retaining a dormant blastocyst for several months in red. Numbers represent average number of days in each stage, according to Poole (1975) and Poole and Catling (1974). Vertical dotted lines indicate the beginning of summer (beige) and winter (grey). The peak in mating takes place in late spring, parturition in early summer, permanent juvenile emergence from the pouch in spring, followed by weaning in winter.

a



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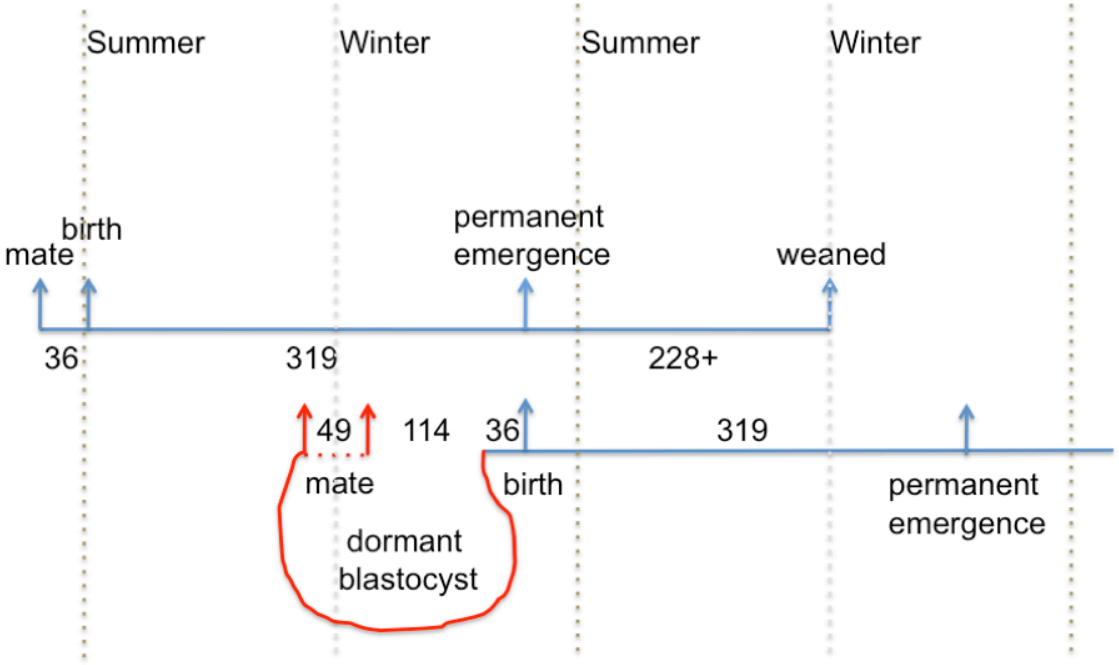
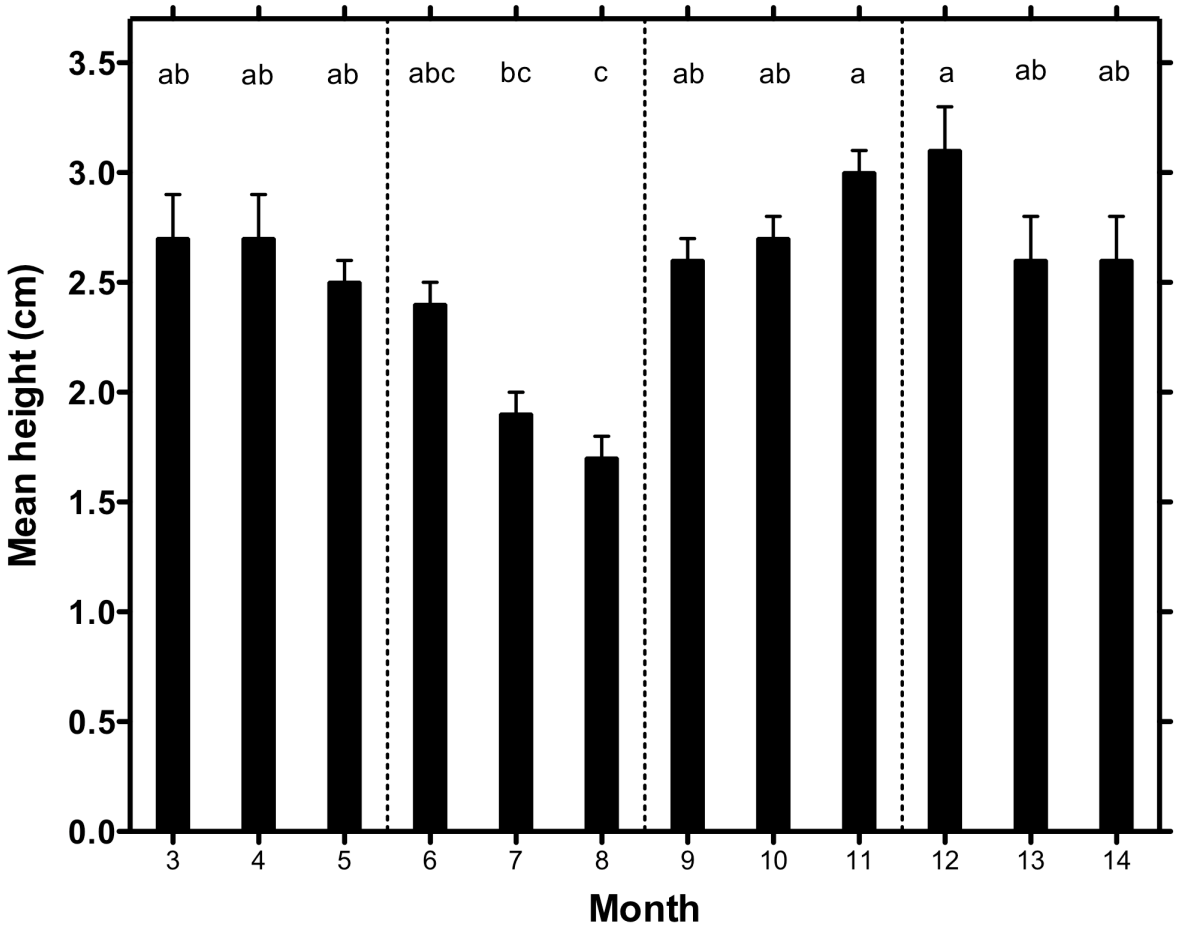


Figure 3. Mean height (± 1SE) of palatable species for eastern grey kangaroos at Wilsons Promontory National Park, Australia, September 2010–August 2012, commencing in autumn (Month 3 = March). Vertical dotted lines denote seasons. Months with the same letter above did not differ according to ANOVA and post-hoc Bonferroni multiple comparison tests (P >0.05).



CHAPTER 2: Grouping patterns show subtle social structure in a species with strong fission-fusion dynamics

Wendy J. King, Graeme Coulson, Marco Festa-Bianchet & Anne W. Goldizen
Manuscript to be submitted to *Behavioral Ecology*.

2.1 ABSTRACT

Eastern grey kangaroos *Macropus giganteus* are gregarious, with females mostly philopatric and most males dispersing. Females are thus suspected to form kin clusters but details of the social structure of this species are unknown. We examined relationships within and between different non-juvenile sex-age classes of kangaroos using half-weight indices and lagged association rates, and calculated six individual network measures using half-weight indices. We found subtle social structure in the marked population. Mean half-weight indices were similar within compared to between different sex-age combinations. Half-weight indices suggested that individuals of all sex-age classes showed preferred and avoided associates. For adult females, there was a weakly positive relationship between pairwise relatedness and dyadic half-weight indices but this effect disappeared after controlling for geographic distance or spatial overlap. Lagged association rates decreased exponentially only for adult males with adult females accompanied by young-at-foot in the breeding season. Most other lagged association rates decreased slightly in a linear fashion and few were constant. Although females without young-at-foot appeared to be more social than females with young-at foot, all associations among individuals were weak. Sample size had strong positive effects on mean half-weight index and all six network measures. We suggest that sample size effects are more likely to occur in large populations where association strengths are generally low and individuals have many weak associations. The social structure of kangaroos is not based on female relatedness at high density under strong fission-fusion dynamics.

2.2 INTRODUCTION

Animals may form complex societies to increase fitness by forming alliances, avoiding potentially dangerous conspecifics, and sharing information about the location of resources (Silk 2007). Although predation pressure can cause animals to aggregate (Lima 1995), complex societies generally involve preferential associations among certain individuals within and between different sex-age classes (Hinde 1976; Whitehead 2008a). In mammals, mother-offspring associations are

persistent because of the need for transfer of milk from mother to young. Consequently, the bonds established among related females during juvenile development may persist into adulthood when females are philopatric (Clutton-Brock and Lukas 2012). Cooperative mammalian societies may thus be based on female kinship (Clutton-Brock 2002). In societies characterised by fission-fusion grouping patterns, benefits to kin associations may be reduced because of the ephemeral nature of groups. Adult female kin have been found to associate preferentially in several species, *e.g.,* African elephants *Loxodonta africana* form family units within larger groups that fuse and break apart from each other within a multi-tiered society (Wittemyer et al. 2005). Kin grouping occurs to a lesser extent in giraffes *Giraffa camelopardalis* (Carter et al. 2013b) and bottlenose dolphins *Tursiops truncatus* (Frère et al. 2010).

Apart from mother-offspring relationships, societies can involve different levels of associations and interactions among other sex-age classes. Assortative interactions can occur if animals associate with conspecifics of similar temperament (Croft et al. 2008) or activity budgets (Ruckstuhl 1998). Because similar-sized animals have equivalent energetic requirements, they may better coordinate feeding and resting activities by associating with each other rather than with different-sized individuals (Conradt 1998). Variation in temperaments and activity budgets can lead individuals to associate with peers of similar age and sex. The tendency to associate with peers is often greater among young animals that are much smaller than adults (Bon and Campan 1996).

Sociality is often made more complex by sexual differences in determinants of fitness (Van Schaik and Kappeler 1997). Reproductive strategies may require that adult males spend much time searching for mates and compete aggressively with other males (Clutton-Brock 1989); thus they might not form long-term associations with individuals of any other sex-age class. In addition, adult females may isolate themselves at parturition to reduce predation risk for young (Bergerud and Page 1987) or to establish the mother-offspring bond at a time when young must learn to identify and follow the mother (Jarman and Southwell 1986). Adult females may form alliances with males to avert infanticide risk from strange males (Ebensperger 1998; Van Schaik and Kappeler 1997).

Macropodids are medium-sized to large marsupial herbivores that are the ecological equivalent of ungulates (Jarman 1983). Social structure and kin-differential behaviour of individually recognisable individuals has been investigated in only a few species of this taxon. Reduced aggression was reported between related female tammar wallabies *Macropus eugenii* in captivity (Blumstein et al. 2002). Although largely solitary, female red-necked wallabies *Macropus rufogriseus* may occasionally associate with matrilineal kin (Johnson 1986). Eastern grey kangaroos *Macropus giganteus* are the most gregarious of macropodids (Jarman 1991), with a fission-fusion social system (Southwell 1984), female philopatry (Zenger et al. 2003) and marked

sexual size dimorphism (Jarman 1989). Males establish dominance hierarchies and display a roving reproductive strategy (Jarman 1994) but dominant males do not appear to influence grouping patterns by excluding other individuals from foraging groups (Jarman 2000). Breeding peaks in the late spring and early summer (Quin 1989). There is a prolonged period of maternal care and lactation lasts for approximately 18 months (Poole 1975). Pouch young emerge permanently from the pouch at approximately 10 months of age and continue to suckle as young-at-foot for a further 8 months (Poole 1975). Kangaroos are an ideal species in which to examine relationships within a fission-fusion system because they form temporary foraging groups during the day and can be habituated to human presence. Females in one study appeared to have non-random associates (Carter et al. 2009), with mean maximum association indices of 0.35 with their closest female associate (AJ Carter, personal communication). Another study found weaker associations among females (mean maximum association indices of 0.22 within communities), which were explained by both home range overlap and kinship (Best et al. 2014; Best et al. 2013). Sub-adults were not distinguished from adults, however, and so the effect of kinship may have been due to the presence of mother-immature daughter pairs. It is thus unclear to what extent females form close associations and whether kinship plays a role in kangaroo society.

Our aim was to quantify association patterns in eastern grey kangaroos. We primarily wished to examine the influence of different sex-age classes (excluding juveniles) on social structure and determine whether non-random associations occurred both within and between sex-age classes. We expected that kinship should influence associations among adult females. Large adult males were expected to be mostly solitary due to their roving reproductive strategy, except for temporary associations between adult males and adult females in the breeding season. Females with dependent young were expected to avoid each other when young permanently emerge from the pouch to establish the mother-offspring bond. Adult females without young-at-foot, sub-adults and small males should all show positive associations with their peers since they likely have similar activity budgets.

2.3 MATERIALS AND METHODS

2.3.1 Study Area and Animals

We studied eastern grey kangaroos at Wilsons Promontory National Park, Australia (38° 57' S, 146° 17' E) from April 2010 to June 2012, as part of a long-term monitoring program that started in 2008. Kangaroos inhabit a 110-ha study area that consists of meadows surrounding a grassy landing strip. The area is mostly open with occasional trees and bushes such as coast tea-tree *Leptospermum laevigatum*, coast wattle *Acacia longifolia* and coast banksia *Banksia integrifolia*

(Davis et al. 2008). Densities of kangaroos were high (approximately 6 individuals/ha) (DM Forsyth, personal communication). There were no wild dogs but predators on juveniles included red foxes *Vulpes vulpes* and wedge-tailed eagles *Aquila audax*.

We captured and marked about 50% of adult females and 80% of adult males following King *et al.* (2011). Adults were marked for visual identification with plastic collars and eartags. Offspring were marked with small plastic eartags, usually while still in the pouch. Animals were aged at first capture according to their mass and reproductive status (presence of pouch young or extended teats in females). Adult females weighed 20–35 kg and adult males 36–63.5 kg. We took ear tissue samples from all captured animals to evaluate genetic relatedness using 9 polymorphic microsatellites (Chapter 3). We estimated birthdates of pouch young based on hind leg, hind foot and head lengths according to Poole et al. (1982). This species reaches sexual maturity in captivity at 2 years in females and 4 years in males (Poole and Catling 1974). Only one female showed signs of reproduction before 3 years of age in this study and she was the only 2-year-old female classed as an adult. A few juveniles were first caught after they had permanently emerged from the pouch but before weaning (young-at-foot) or after weaning (sub-adults) and were given eartags but not collars. Captures were undertaken with ethics approval from the University of Melbourne (#0810628.1 and #0911512.1) and research permits from the Victorian Department of Sustainability and Environment (#1004582 and #1005558). Observations were conducted with animal ethics approval from the University of Queensland (#SIB/206/09/(NF)).

Kangaroos have two daily peaks in activity (Clarke et al. 1989) and so we observed them while they were foraging for 2–3 h after dawn and before dusk using 8X32 binoculars (Leitz, Germany). Observations involved one observer (WJK) walking slowly around sections of the study area for 10 to 12 consecutive days each month between April 2010 and June 2012 (total 1,122 h). A second observer (MFB) attempted captures in another section of the study area at the same times from July 2010 to August 2011, in October 2011 and in March 2012 and took additional observations only if the animals were undisturbed, providing approximately 25% of the observations. Kangaroos were habituated to humans in this part of the park and our observations usually did not disturb feeding kangaroos. We did not conduct observations if kangaroos bunched up and moved away from us or if they were resting. It was impossible to determine group compositions while animals were resting because kangaroos were often partially hidden by vegetation and also tended to be disturbed by human approach. Resting patterns could reflect different association patterns from active patterns, however kangaroos appear to seldom interact while resting. We recorded individuals' locations from a distance of approximately 15 m using a hand-held Global Positioning System unit (GPSmap 60Cx, Garmin, Olathe, KS) with a precision of 4 m, and adjusted for observer/animal distance using a range-finder (SCOUT1000, Bushnell,

Lenexa, KS) and compass (KB-14/360R, Suunto, Keili, Finland). We also recorded group size (including unmarked individuals) and group composition of marked individuals. Because the entire area could not be surveyed in one observation session, different sections of the study area were surveyed in the morning and the evening on a rotational basis, with the 'main' section of the area being visited almost every day (Figure 1).

Animals entered the dataset when newly marked, which mostly occurred in July through September for adult females. Observations of marked pouch young were recorded whenever they were out of the pouch. Most pouch young permanently left the pouch and became 'young-at-foot' from October through December. Adult males were marked throughout the year, whenever they first appeared on the study area. Animals left the dataset through emigration and mortality, the latter of which peaked in the winter months of July and August (WJ King, personal observation). For these reasons and to maximise the number of individuals followed, we restricted evaluation of associations to October 2010–June 2011, including only those present for the entire time period of 245 days. Since young-at-foot associate closely with their mothers, their associations likely depend directly on their mother's associations (Jarman 1994) and so we excluded this age group from analyses. Sub-adults were about 2–2.5 years old in October-June. Males were divided by size into sub-adult males (2-year-olds), small males (approximately the size of adult females and 3 or 4 years of age) and large adult males (larger than adult females and at least 5 years old). Adult females that had young-at-foot were classed separately from adult females without young-at-foot.

2.3.2 Analyses

We calculated half-weight indices (HWIs) according to daily group compositions in SOCPROG 2.4 (Whitehead 2009) and defined a group using the 10-m 'chain rule', *i. e.* the most peripheral member of a group was within 10 m of at least one other group member (Jarman 1987). Following Clutton-Brock et al. (1982), we chose this distance based on preliminary observations on 3 days in February 2010 that showed a distinct discontinuity in nearest-neighbour distances beyond that point. Within groups, 87% of nearest-neighbour distances were less than 7 m (Figure 2). Our definition of group reflects active associations and the likelihood that individuals will interact, according to the 'gambit of the group' (Whitehead and Dufault 1999). Individuals had to be seen at least 10 times to be included in analyses. We chose the HWI as the association index because it corrects for a bias when not all associates of an individual are identified due to individuals being observed for only a small proportion of the sampling period (Whitehead 2008a). We calculated a centroid for each individual seen at least 10 times as the mean x and y co-ordinates of sightings. We also calculated activity centres based on 95% kernels in Ranges8 (Kenward et al. 2008) using the fixed kernel method (Worton 1989) and a smoothing factor h of 0.63, which was obtained as the

median using least-squares cross-validation (Kenward 2001) for individuals seen at least 30 times (Seaman et al. 1999). We then examined dyadic spatial overlap as the percentage overlap of 95% kernels, calculated separately for each pair member. Sizes of 95% kernels were logarithmically transformed and compared for females with young-at-foot to those of females without young-at-foot using *t*-tests.

We initially utilised the commonly used Bejder et al. (1998) randomisation method (Whitehead 2008a) to determine whether preferred associations existed in the population. Where the coefficient of variation (CV) of the observed association indices was greater than that of the random indices using 1,000 permutations, we concluded that at least some pairs showed social preferences; we did this for all pairs combined and also for different sex-age classes. Similarly, the presence of avoided associations was detected by comparing the proportion of observed non-zero indices to that generated from 1,000 permutations. This method accounts for how often individuals are found in different sized groups by sequentially flipping observations of individuals between groups while holding constant both the number of groups and group size. Because the number of times an animal is seen is held constant, randomisation tests control for variation in sample size (Croft et al. 2008).

We evaluated the extent of social complexity or social differentiation, *S*, in the population using the coefficient of variation of the true association indices and the maximum likelihood approximation (Whitehead 2008b). *S* varies from 0 in homogeneous societies to > 10 in extremely differentiated societies and is ~ 0.8 in highly differentiated societies (Whitehead 2008a). We also used principal component analysis (PCA) to determine how much of the variance in HWIs could be explained by the first 2 eigenvectors of the association matrix, and thus whether there was significant social structure overall (Whitehead and Dufault 1999). The distance between two points (or individuals) in the PCA plot is inversely related to the dyadic HWI, and clusters of points indicate sets of individuals that associate highly (Whitehead 2008a).

We used a Mantel (1967) test with 1,000 permutations to determine whether there was differential association within compared to between sex-age classes (Whitehead 2008a). We also used Mantel tests to determine the strength of the correlation between association indices and geographic distances and spatial overlap, and whether association indices between pairs of adult females were correlated with pairwise relatedness *r*, as calculated in KINGROUP (Konovalov et al. 2004). We then controlled for geographic distance and spatial overlap in the latter correlation using partial Mantel tests. We used ANOVA followed by Bonferroni *post-hoc* tests to investigate variation in the proportion of time individuals of different sex-age classes were found alone. Females alone with their young-at-foot-were considered to be alone.

We calculated five network measures for individuals using SOCPROG 2.4 (Whitehead 2009): strength, eigenvector centrality, reach, clustering coefficient and affinity. Strength is simply the sum of the HWIs, is equivalent to typical group size (the group size an individual usually finds itself in (Jarman 1974)) minus 1, and measures gregariousness. Eigenvector centrality measures the connectedness of an individual within a network and we used the first eigenvector method of Newman (2004). Reach measures the overall strength of an individual's associates two or fewer steps away. The clustering coefficient measures the association of an individual's associates and we used the formula of Holme et al. (2007). Affinity is simply reach divided by strength and therefore measures the mean strength of associates relative to that of the individual. Because both strength and affinity use the sum of an individual's HWIs in their calculations, they are likely to be correlated. We also determined a sixth network measure, degree; the number of associates for an individual. These six network measures were proposed by Whitehead (2008a), with commonly used measures being degree, strength, eigenvector centrality and the clustering coefficient (Wey et al. 2008).

Recently, there have been warnings of the tendency for commonly used association indices (such as HWIs) to overestimate specific social preferences between pairs of individuals in situations with large variation in gregariousness among individuals (Godde et al. 2013). However, the tendency for variation in individual sample size to influence the number of associates, the mean association indices, and individual network measures based on those indices appears to have been overlooked. Most authors set a minimum number of observations per individual to avoid rarely seen animals having a disproportionate effect on results (Whitehead 2008a), but the possible effect of individual sample size on individual association measures has not been examined. To investigate the relationship between sample size and individual measures of sociability, we compared Pearson correlations based on a minimum of 10 or 40 observations.

2.3.3 Lagged Association Rates

To measure temporal patterns and stability of preferred associations, we calculated lagged association rates (LARs) in SOCPROG 2.4 (Whitehead 2009). LARs evaluate rates of re-association following an initial pairwise association. We fitted models that included rapid disassociation within one sampling period (a day) followed by persistent associations (LAR2; 1 parameter), linear decrease or increase in associations (custom models; 2 parameters), exponential decay in associations (LAR4; 2 parameters), exponential decay in associations that leveled off (LAR6; 3 parameters) and two periods of exponential decay (LAR8; 4 parameters) (Whitehead 2008a). We used 1 day as the time lag because we used 1 day as the sampling period for

association indices. Kangaroo groups form and fission several times an hour (Jarman 2000) so there was ample opportunity for individuals to form new associations within the chosen time lag. We compared models using quasi Akaike information criteria (QAICs) and chose the best fit by minimising QAIC (Whitehead 2007) and parameters (Burnham and Anderson 2004), although we also considered models with $\Delta QAIC < 2$ as good fits to the data. When linear custom models gave similar results to LAR4 exponential models, we preferred the former because they were simpler. When associations appeared to be cyclic, we also fitted cosine (3 parameter) custom models and compared them to LAR2, LAR4, LAR6 and LAR8 models. We calculated null models as expected LARs based on random association patterns. Models with exponential decay can be used to determine the time period for temporary associations (1/exponent) and associations occurring well above the null association rate are indicative of preferred associations (Whitehead 2008a). We calculated standard errors using jackknifing procedures over 2 days (Whitehead 2007).

There are two types of informative results from LARs. The first is the shape of the curve because it shows how the associations vary with time. A flat line indicates that pairs consistently associate, or that some pairs re-associate after a time lag while others disassociate at the same rate. A descending line shows disassociations while an increasing line shows re-associations among pairs. Cyclic associations can be determined from curves that incorporate cosine relationships. Although Whitehead (2008a) used the terminology 'constant companions' and 'casual acquaintances' to describe the shapes of the various curves, different social structures can result in similar forms of the LAR curves (Whitehead 2008a). As described above, a flat line may not represent a persistent association, yet it would fit the 'constant companion' type of curve. Also, a line that was no different from the null association rate would still be termed as fitting the 'constant companion' model. We thus refrain from using these terms and describe the curves in terms of their mathematical equations. The second informative result of LAR analyses is the elevation of the curve and its standard error with respect to the null association rate. Preferred associations can be inferred from lines with standard errors that do not overlap those of the null.

2.4 RESULTS

2.4.1 General Social Structure

The social structure of eastern grey kangaroos in our high-density population can be described using a variety of measures based on association indices (HWIs; Table 1). There were 153 marked individuals present for the entire period October 2010–June 2011, over which we recorded 6,737 groups that varied in size from 1–40, although 99% of groups consisted of 1–12 individuals. Mean proportion of marked individuals identified on any one of the 122 sampling days

was 0.44. Each individual had an average of 45 marked associates (Table 1). Mean HWI was extremely low at 0.010 ± 0.005 and mean maximum HWI was also low at 0.122 ± 0.090 (Table 1). Typical group size (strength plus 1) was 2.4, however individuals showed both preferred and avoided associations across the study area (Table 1). Social differentiation *S* was fairly high at 0.81 but the correlation between the true and the estimated association indices was low at 0.36.

Only 3.0% of the variance in HWIs could be explained by the first 2 factors in PCA, indicating little social structure. Individuals did not cluster in the two plot dimensions but were distributed along two axes (Figure 3). The first factor appeared to be related to location within the study area and we found a strong negative relationship between geographic distance and HWI, whether we used the distance between centroids (Mantel test, $r = -0.41$, $P = 1.000$, $n = 153$ individuals; Figure 4) or distance between 95% kernel centres (Mantel test, $r = -0.39$, $P = 1.000$, $n = 142$). Similarly, HWI was positively correlated with overlap of 95% kernels (Mantel test, $r = 0.45$, $P = 0.001$, $n = 142$ individuals). No dyads that had centroids farther than 275 m apart had association indices above 0.10, and the highest association index (0.48) was between a mother and her sub-adult son that had centroids 29 m apart (Figure 4).

Mean HWIs were similar within compared to between different sex-age combinations (Mantel test, $P = 0.95$), although higher values seemed to involve sub-adults of both sexes and small males while lower values appeared to involve large adult males and adult females with young-at-foot (Table 2). Maximum HWIs by sex-age class also seemed to follow the same pattern (Table 3). For adult females, there was a weakly positive relationship between pairwise relatedness and HWIs, yet closely related adult females did not have high association indices (Mantel test, $r = 0.05$, $P = 0.013$, $n = 76$ females; Figure 5). None of the pairs of adult females with estimated relatedness above 0.4 had HWIs over 0.06 (Figure 5). After controlling for geographic distance between centroids or 95% kernel centres of dyads, there was no relationship between relatedness and HWIs (partial Mantel tests, $r = 0.02$, $P = 0.23$, $n = 76$ or $r = 0.02$, $P = 0.19$, $n = 75$ females). Mean proportion time spent with the closest adult female associate was 0.073 ± 0.030 and ranged from 0.023 to 0.150, based on maximum HWIs among adult females.

There was significant variation in the amount of time that different sex-age classes were alone (ANOVA, $F_{5,147} = 20.06$, $P = 0.001$). Adult females with young-at-foot were more often alone than adult females without young-at-foot and large adult males were also often alone (Figure 6). Adult females accompanied by young-at-foot also had smaller ranges and core areas compared to females without young-at-foot (ranges: 12.4 ± 0.7 ha *vs.* 16.4 ± 1.5 ha, $t_{51,22} = 2.50$, $P = 0.015$; cores: 4.4 ± 0.3 ha *vs.* 6.0 ± 0.7 ha, $t_{51,22} = 2.04$, $P = 0.045$).

2.4.2 Effects of Sample Size on Individual Network Measures

The number of observations per individual varied according to sex-age class (ANOVA, $F_{5,147} = 2.40$, $P = 0.040$; Table 1). Of the seven measures of sociability that we investigated, all but the clustering coefficient were highly and positively correlated with sample size (Table 5, Figures 7-10). When a minimum of 40 observations was used, the clustering coefficient also became highly related to sample size (Table 5). Overall, an increase from 50 to 100 observations doubled the mean HWI from 0.007 to 0.014 across all sex-age classes (Figure 7). The slope of the correlation between number of associates and sample size was 0.55; an individual was seen with a new associate every 2 observations on average (Figure 8). Strength, centrality, reach and the clustering coefficient all approximately doubled with an increase from 50 to 100 observations (Table 5, Figures 9, 10) whereas affinity increased by a factor of 1.2 (Table 5, Figure 9).

2.4.3 Temporal Patterns of Associations

The LARs revealed seven temporal patterns of associations (Supplementary Figures 1-21). The most common relationships were slight linear decreases following rapid disassociation within a day, so that the mean likelihood of a pair being seen together after a time lag of 1 day was low and varied from 0.022 to 0.127 (Table 6: Supplementary Figures 1-3, 7, 9, 11-13, 18). Slopes of the lines were also shallow, ranging from 0.000017 to 0.00023 (Table 6). Exponential decay models fitted the data better than linear models according to QAICs only for adult males with adult females accompanied by young-at-foot (Table 6; Supplementary Figure 4). The time period for exponential decay for that sex-age combination was estimated to be 1.1 day (Table 6). Sub-adult females were the only sex-age combination showing a slight linear increase in associations with time following rapid disassociation (Table 6; Supplementary Figure 15). A few relationships were constant following rapid disassociation, but because they occurred at low levels (0.020 to 0.060; Table 6: Supplementary Figures 5, 8, 20), they likely resulted from some pairs re-associating after a time lag while others disassociated at the same rate, rather than from persistent associations among pairs. Several sex-age combinations showed slight quadratic increases followed by decreases (Supplementary Figures 10, 19, 21), or the reverse (Supplementary Figures 6, 16) and one combination showed a weak exponential increase followed by a decrease (Supplementary Figure14) with rapid disassociation. The last association pattern was cyclic and involved rapid disassociation followed by cyclic decreases then increases (Table 6, Supplementary Figure 17). This pattern was seen only for sub-adult males with adult females without young-at-foot, with a periodicity of 131 days (Table 6). Observations began at the beginning of the breeding season and the increases in association rate occurred after the peak in breeding (Supplementary Figure 17).

All LARs were above the null models, which seemed to indicate preferred associations for all sex-age combinations, however only a few combinations associated at levels that were considerably higher than the null rates (Table 6). The highest levels of associations (*i.e.*, LARs > 0.10) were among small males (Supplementary Figure 10) and for both sub-adult females and males with adult females without young-at-foot (Supplementary Figures 4 and 5, Table 6). Both sexes of sub-adults associated more with this class of adult female than with adult females accompanied by young-at-foot (Supplementary Figures 11-12, 16-17). Adult females without young-at-foot showed a slightly higher level of association among themselves (Supplementary Figure 3) than did females accompanied by young-at-foot (Supplementary Figure 1).

2.5 DISCUSSION

We found little social structure in eastern grey kangaroos both when using association indices and lagged association rates. The weak relationship between association indices and pairwise relatedness among adult females disappeared after controlling for geographic distance or spatial overlap between dyads. Another study of kangaroos found that females preferred to associate with other females with which they had a mean relatedness of 0.05 ± SD 0.22, after controlling for range overlap (Best et al. 2014). Mantels tests comparing relatedness to association indices showed weak correlation ($r = 0.13$) (Best et al. 2014). Groups were defined using a larger chain rule (15-m compared to 10-m) and thus would have been more likely to encompass more individuals; typical group sizes were larger than in the present study (4.3 compared to 2.4) (Best et al. 2013). The weak effect of kinship on preferred associations, however, was more likely influenced by inclusion of mother/sub-adult daughter pairs, as sub-adults were not distinguished from adults. In an earlier study conducted on a population at lower density (approximately 0.4–0.7 kangaroos/ha), about 60% of females formed close associations with other adult females that were matrilineal kin (Jarman 1994), but bi-parental genetic relationships were not known. Kinship played no role in associations among adult females in our study. Females may form close associations with related adult females in low-density populations, simply because they are gregarious and have few options for potential associates. It is clear, however, that in our high-density population, females do not choose to associate with close kin.

Females with young-at-foot tended to isolate themselves from other kangaroos. For all large macropod species, the basic social group is a mother with a young-at-foot. This association type is prevalent in *M. giganteus* (Jaremovic and Croft 1991; Jarman 1994; Kirkpatrick 1966; Southwell 1984), *M. fuliginosus* (Coulson 1993; Johnson 1983), *M. rufus* (Croft 1981a; Johnson 1983), *M. robustus* (Croft 1981b; Taylor 1983) and *M. antilopinus* (Croft 1982). Females may isolate

themselves to prevent the juvenile from being separated from its mother when disturbance causes flight or because the offspring has limited locomotory ability (Croft 1981a). Southwell (1984) showed that fission-fusion rates increased exponentially with density in eastern grey kangaroos but that the tendency for females to isolate themselves was constant over densities of 0.1–1.0 animals/ha. He concluded that group formation was loose and flexible, in support of previous studies (Caughley 1964), except for the associations of mothers with young-at-foot and adult males with oestrous females. We found similar results, with adult males also tending to be somewhat solitary. Males need to search for mates to maximise reproductive success (Jarman and Southwell 1986). The reasons why male and female adults should choose to be solitary are thus different for the two sexes. It is noteworthy, however, that adult females accompanied by young-at-foot isolated themselves from groups even at the very high density of approximately 6 animals/ha. This behaviour must therefore be an important component of maternal care.

Sample size had a strong positive effect on mean HWI and all six network measures in our study. This was likely a result of mean association indices being very low (0.01) but researchers should investigate this possibility in other species with strong fission-fusion dynamics. Although mean HWIs for individual kangaroos were influenced by sample size, pairwise HWIs should still accurately estimate proportions of time spent together for dyads. The positive effect of individual sample size on network measures is more likely to be strong in large populations in which association strengths are generally low and individuals have many weak associations. Species that potentially fit these criteria are those that frequently form small subgroups within large aggregations, such as open country ungulates or shoaling fish. African buffalo *Syncerus caffer* form aggregations that fission and fuse but group sizes are large (often >200 individuals) and fission events are rare (approximately 1/day) (Cross et al. 2005). Giraffes form small groups of about 4 individuals (Carter et al. 2013b) but female-female association rates of about 0.3 are stable across time (Carter et al. 2013a). Shoaling fish such as Trinidadian guppies *Poecilia reticulata* frequently form small groups of about 5 individuals and fission events occur approximately every 10 secs (Croft et al. 2003), however 'association strengths' of some female-female pairs are 0.3–0.4 (Croft et al. 2004). We have thus found few published studies of social structure where recognisable individuals associate at such low levels as in our study of kangaroos, possibly due to the logistic difficulties of undertaking such studies. Northern bottlenose whales *Hyperoodon ampullatus*, and bottlenose dolphins, however, appear to fit the above criterion of low mean association indices (0.01–0.02) and high fission-fusion dynamics (Frère et al. 2010; Gowans et al. 2001). Using individual network measures such as degree, strength, centrality, reach, clustering coefficient and affinity could be problematic in further correlation analyses with phenotypic characteristics (*e.g.* sex, size, parasite load and personality) or reproductive success, without controlling for

observational sample size of individuals. We suggest that researchers verify whether sample size has an effect on individual network measures in other species.

Whitehead (2008a) points out that lagged association rates, unlike association indices, are valid with small sample sizes for each individual. We show that lagged association rates can be useful to interpret social structure when large sample sizes inflate mean association indices. Whitehead (2008a) recommends including all observations of animals in lagged association analyses because they can delineate mortality and emigration. However, if one knows when animals die and disperse, then changes in lagged association rates indicate changes in the strength of associations of animals still present in the study.

The lagged association rate analyses showed subtle patterns of associations between and among different sex-age classes of kangaroos. As expected, females without young-at-foot, sub-adults and small males showed preferential associations with peers, although all associations were weak (<0.13). Adult males appeared less social than all other sex-age classes and adult females accompanied by young-at-foot were less social than adult females without young-at-foot. Lagged association rates decreased exponentially only for adult males with adult females accompanied by young-at-foot, lasting only for 1 day in the breeding season. Lagged association rates show pair bonds in bottlenose dolphins and killer whales *Orcinus orca* that can last several years (Baird and Whitehead 2000; Lusseau et al. 2003; Owen et al. 2002; Wiszniewski et al. 2009). Initial association rates in those species started at around 0.35–0.7 and remained well above null association values of 0.0–0.25 (Baird and Whitehead 2000; Lusseau et al. 2003; Owen et al. 2002; Wiszniewski et al. 2009). Strong persistent associations have also been shown in leaf-roosting bats *Thyroptera tricolor* (0.9–0.4) and nesting southern flying squirrels *Glaucomys volans* (0.75–0.4) (Garroway et al. 2013; Vonhof et al. 2004). In contrast, weaker associations have been detected in giraffes, where lagged association rates fell from 0.3 to 0.1 after around 100 days (Carter et al. 2013a). We conclude that since lagged association rates below 0.1 indicate a less than 10% probability that pairs will associate after a specified time lag, rates below 0.1 indicate weak associations, which was the level of most of the initial lagged association rates in our study.

The social structure of eastern grey kangaroos that we describe here is unlike that found in other large herbivores where adult females tend to form either stable family groups, as in white-tailed deer *Odocoileus virginianus* and red deer *Cervus elaphus* (Aycrigg and Porter 1997; Clutton-Brock et al. 1982) or more temporary groupings of mostly unrelated individuals, as in bighorn sheep *Ovis canadensis* and mountain goats *Oreamnos americanus* (Festa-Bianchet 1991; Festa-Bianchet and Côté 2008). We found that female kangaroos had very ephemeral associations and that the only social bonds outside the mother/ juvenile offspring pair were between adult males and adult females in the breeding season. Similar subtle social structure involving male-female liasons

but not female-female associations has been described in quokkas *Setonix brachyurus,* a medium-sized macropodid (McLean et al. 2009). It is likely that predation pressure is the most important factor driving grouping in macropodids and that social factors such as kinship do not confer benefits. Indeed, close associations between red-necked wallaby *Macropus rufogriseus* females and their sub-adult daughters can lead to reduced reproductive success (Johnson 1986).

2.6 ACKNOWLEDGEMENTS

This work was supported by the Natural Sciences and Research Council of Canada (postgraduate scholarship to WJK and operating grant to MFB), the Holsworth Wildlife Research Endowment and the Senate Research Committee of Bishop's University. U Gélin and E Hynes assisted with captures. J Chambers and E Rioux-Paquette conducted the microsatellite screening for this project.

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Table 1. Attributes of the social structure of marked eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. Standard errors are shown in parentheses and are derived from 1,000 bootstrap replicates for HWIs and network measures. *P*-levels < 0.05 or > 0.95 indicate avoided or preferred associations, respectively and are highlighted in bold. AFY = adult female with a young-at-foot; AFN = adult female without a young-at-foot; AM = large adult male; SM = small male; SAF = sub-adult female; SAM = sub-adult male.

Attribute	Overall	AFY	AFN	AM	SM	SAF	SAM
No. individuals	153	54	23	26	7	17	26
Mean no. observations	67.0 (1.7)	71.2 (2.8)	72.5 (4.0)	59.5 (4.8)	57.7 (9.3)	72.0 (4.4)	60.5 (3.9)
Mean no. associates	44.6 (1.5)	43.6 (2.4)	48.0 (3.9)	36.4 (3.9)	44.4 (8.6)	50.0 (3.5)	48.7 (4.3)
Mean HWI	0.010 (0.005)	0.008 (0.004)	0.010 (0.004)	0.007 (0.004)	0.011 (0.007)	0.013 (0.004)	0.012 (0.005)
Mean maximum HWI	0.122 (0.090)	0.094 (0.059)	0.166 (0.126)	0.080 (0.026)	0.113 (0.037)	0.182 (0.099)	0.147 (0.110)
Prop. non-zero elements							
- observed	**0.29**	**0.29**	**0.32**	**0.24**	**0.29**	**0.33**	**0.32**
- random	**0.33**	**0.34**	**0.39**	**0.28**	**0.32**	**0.43**	**0.40**
- *P*	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
CV of HWIs- observed	**2.24**	**2.12**	**2.32**	**2.17**	**1.99**	**2.17**	**2.19**
- random	**1.88**	**1.70**	**1.55**	**1.83**	**1.88**	**1.51**	**1.58**
- *P*	**1.000**	**1.000**	**0.999**	**1.000**	**1.000**	**0.999**	**1.000**
Mean strength	1.45 (0.08)	1.27 (0.09)	1.57 (0.09)	1.05 (0.07)	1.62 (0.20)	1.94 (0.11)	1.77 (0.12)
Mean eigenvector centrality	0.065 (0.003)	0.057 (0.004)	0.065 (0.006)	0.041 (0.004)	0.082 (0.011)	0.090 (0.008)	0.085 (0.006)
Mean reach	2.62 (0.34)	2.31 (0.34)	2.80 (0.35)	1.68 (0.21)	2.97 (0.65)	3.63 (0.42)	3.28 (0.45)
Mean clustering coefficient	0.055 (0.008)	0.059 (0.009)	0.056 (0.009)	0.042 (0.007)	0.049 (0.009)	0.063 (0.009)	0.057 (0.009)
Mean affinity	1.71 (0.10)	1.74 (0.12)	1.71 (0.10)	1.52 (0.10)	1.69 (0.13)	1.83 (0.11)	1.73 (0.10)

Table 2. Mean HWIs for different combinations of 6 sex-age classes of 153 marked eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. Standard errors are shown in parentheses and are derived from 1,000 bootstrap replicates. AFY = adult female with a young-at-foot; AFN = adult female without a young-at-foot; AM = large adult male; SM = small male; SAF = sub-adult female; SAM = sub-adult male.

Sex-age class	AFY	AFN	AM	SM	SAF	SAM
AFY (*n* = 54)	0.008 (0.004)					
AFN (*n* = 23)	0.009 (0.005)	0.011 (0.005)				
AM (*n* = 26)	0.006 (0.003)	0.006 (0.004)	0.011 (0.005)			
SM (*n* = 7)	0.009 (0.006)	0.008 (0.007)	0.010 (0.005)	0.024 (0.021)		
SAF (*n* = 17)	0.012 (0.005)	0.017 (0.008)	0.006 (0.003)	0.011 (0.008)	0.016 (0.007)	
SAM (*n* = 26)	0.010 (0.005)	0.013 (0.008)	0.007 (0.003)	0.014 (0.010)	0.016 (0.010)	0.017 (0.011)

Table 3. Mean maximum HWIs for different combinations of 6 sex-age classes of 153 marked eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. Standard errors are shown in parentheses and are derived from 1,000 bootstrap replicates. AFY = adult female with a young-at-foot; AFN = adult female without a young-at-foot; AM = large adult male; SM = small male; SAF = sub-adult female; SAM = sub-adult male.

Sex-age class	AFY	AFN	AM	SM	SAF	SAM
AFY (*n* = 54)	0.063 (0.022)					
AFN (*n* = 23)	0.064 (0.034)	0.069 (0.040)				
AM (*n* = 26)	0.052 (0.023)	0.043 (0.020)	0.067 (0.026)			
SM (*n* = 7)	0.067 (0.029)	0.056 (0.033)	0.081 (0.031)	0.082 (0.058)		
SAF (*n* = 17)	0.108 (0.074)	0.136 (0.108)	0.047 (0.021)	0.044 (0.022)	0.089 (0.052)	
SAM (*n* = 26)	0.091 (0.063)	0.093 (0.110)	0.048 (0.021)	0.047 (0.031)	0.088 (0.057)	0.089 (0.048)

Table 4. Pearson correlation coefficients (r), P-values and equations for relationships between sample size and several measures of sociability for 153 eastern grey kangaroos seen a minimum of 10 times and 136 kangaroos seen a minimum of 40 times at Wilsons Promontory National Park, Australia, October 2010 to June 2011.

		Minimum 10 observations			Minimum 40 observations	
Measure	Equation	r	P	Equation	r	P
Mean HWI	y=0.00014x-0.0002	0.66	0.001	y=0.00013x+0.0011	0.49	0.001
No. of associates	y=0.55x+7.5	0.62	0.001	y=0.38x+20.9	0.37	0.001
Strength	y=0.022x-0.01	0.66	0.001	y=0.019x-0.18	0.49	0.001
Eigenvector centrality	y=0.00095x+0.001	0.42	0.001	y=0.00072x+0.019	0.24	0.004
Reach	y=0.043x-0.27	0.61	0.001	y=0.040x-0.05	0.46	0.001
Clustering coefficient	y=0.00019x+0.042	0.14	0.09	y=0.00069x+0.004	0.44	0.001
Affinity	y=0.0054x+1.34	0.40	0.001	y=0.0061x+1.29	0.36	0.001

Table 5. Lagged association rate models fitted to 21 sex-age class combinations of 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. AFY = adult female with a young-at-foot; AFN = adult female without a young-at-foot; AM = large adult male; SM = small male; SAF = sub-adult female; SAM = sub-adult male. Time lag is τ in days.

Sex-age class combination	General shape	Formula	Value at τ =1	Value at τ =200	Null	Above null at τ?
AFY- AFY	linear decrease	$g=0.022-0.000017\,\tau$	0.022	0.019	0.010	yes
AFN- AFY	linear decrease	$g=0.034-0.000070\,\tau$	0.034	0.020	0.010	yes
AFN- AFN	linear decrease	$g=0.049-0.00010\,\tau$	0.049	0.029	0.015	yes
AM- AFY	exponential decrease, constant	$g=0.014+0.123e^{-0.89\,\tau}$	0.064	0.014	0.005	yes to 3
AM- AFN	constant	$g=0.020$	0.020	0.020	0.010	yes
AM- AM	quadratic decrease, increase	$g=0.025-0.00014\,\tau+0.00000079\,\tau^2$	0.025	0.029	0.010	yes
SM- AFY	linear decrease	$g=0.034-0.00011\,\tau$	0.034	0.012	0.010	yes to 100
SM- AFN	constant	$g=0.037$	0.037	0.037	0.015	yes at 100
SM- AM	linear decrease	$g=0.035-0.000035\,\tau$	0.035	0.028	0.010	yes
SM- SM	quadratic increase, decrease	$g=0.106+0.00030\,\tau-0.0000047\,\tau^2$	0.106	0.000	0.030	yes to 100
SAF- AFY	linear decrease	$g=0.067-0.000080\,\tau$	0.067	0.051	0.010	yes
SAF- AFN	linear decrease	$g=0.127-0.00023\,\tau$	0.127	0.081	0.020	yes
SAF- AM	linear decrease	$g=0.027-0.000070\,\tau$	0.027	0.013	0.005	yes to 100
SAF- SM	exponential increase, decrease	$g=-0.204e^{-0.024\,\tau}+0.212e^{-0.015\,\tau}$	0.010	0.009	0.010	yes at 50
SAF- SAF	linear increase	$g=0.057+0.000094\,\tau$	0.057	0.076	0.015	yes
SAM- AFY	quadratic decrease, increase	$g=0.062-0.00034\,\tau+0.0000013\,\tau^2$	0.062	0.046	0.010	yes
SAM- AFN	2X cyclic decrease, increase	$g=0.018\cos(0.048\tau)+0.084$	0.102	0.066	0.015	yes
SAM- AM	linear decrease	$g=0.023-0.000064\tau$	0.023	0.010	0.005	yes to 100
SAM- SM	quadratic increase, decrease	$g=0.035+0.000090\,\tau-0.0000010\,\tau^2$	0.035	0.013	0.020	yes to 50
SAM- SAF	constant	$g=0.060$	0.060	0.060	0.020	yes
SAM- SAM	quadratic increase, decrease	$g=0.057+0.00024\,\tau-0.0000015\,\tau^2$	0.057	0.045	0.020	yes

Figure 1. Aerial photo of the study area at Wilsons Promontory National Park, Australia, showing the 'main' area observed outlined in yellow and the entire area surveyed in red. The photo was taken 25 August 2011.

Figure 2. Distribution of nearest-neighbour distances for eastern grey kangaroos at Wilsons Promontory National Park, Australia, April 2010 to June 2012 (n = 21,110).

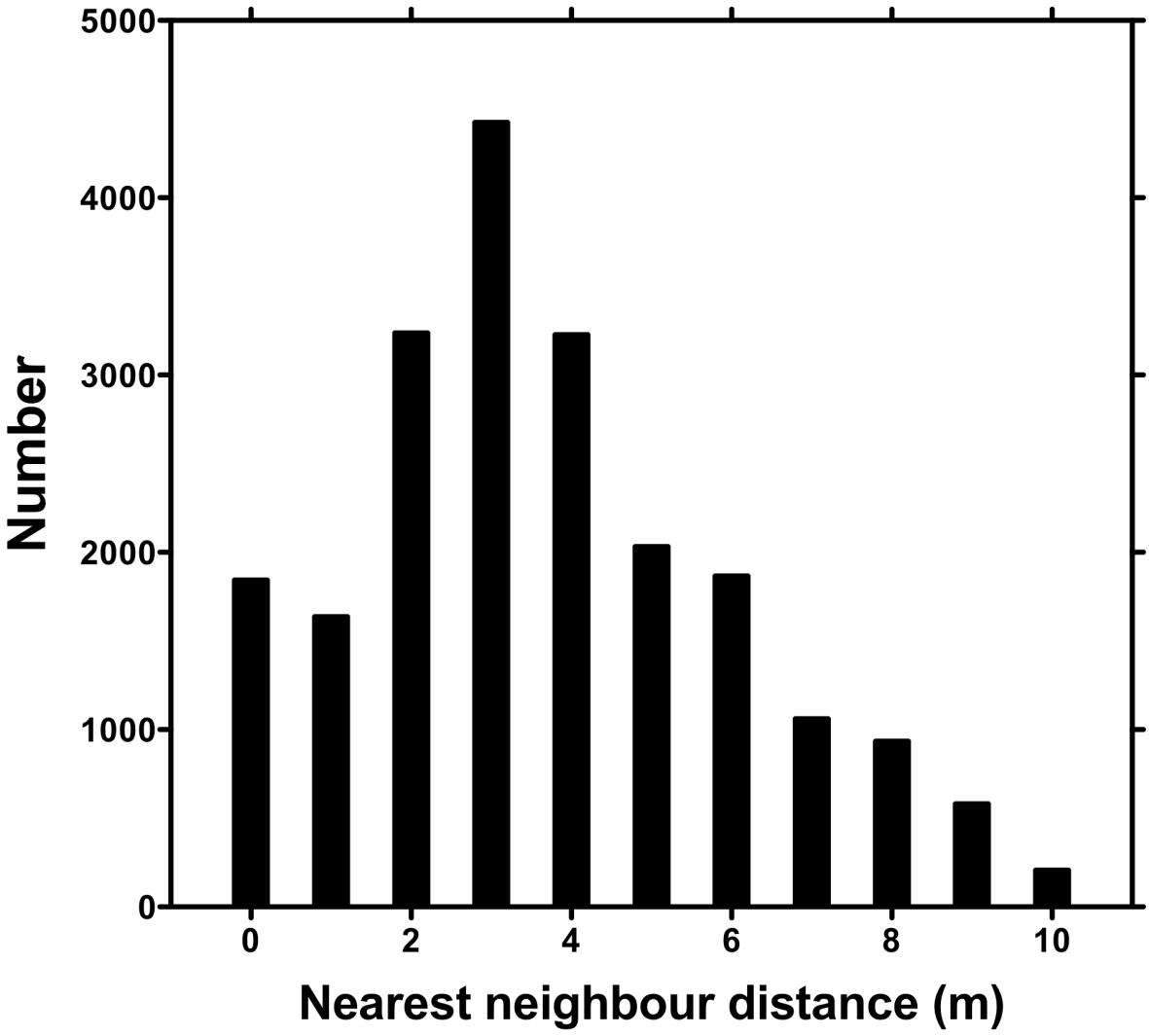


Figure 3. Principal component analysis of HWI values for 153 eastern grey kangaroos at Wilsons' Promontory National Park, Australia, October 2010 to June 2011.

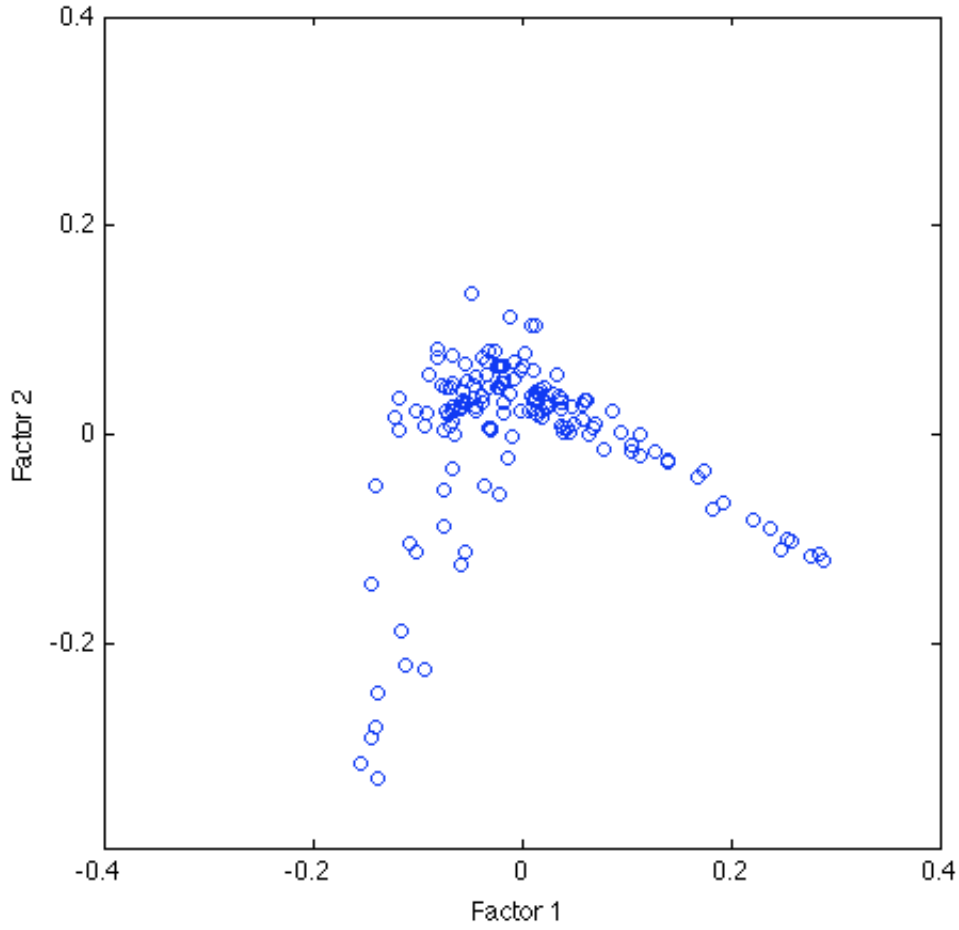


Figure 4. Relationship between geographic distance and HWI for 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011 (n = 11,628).

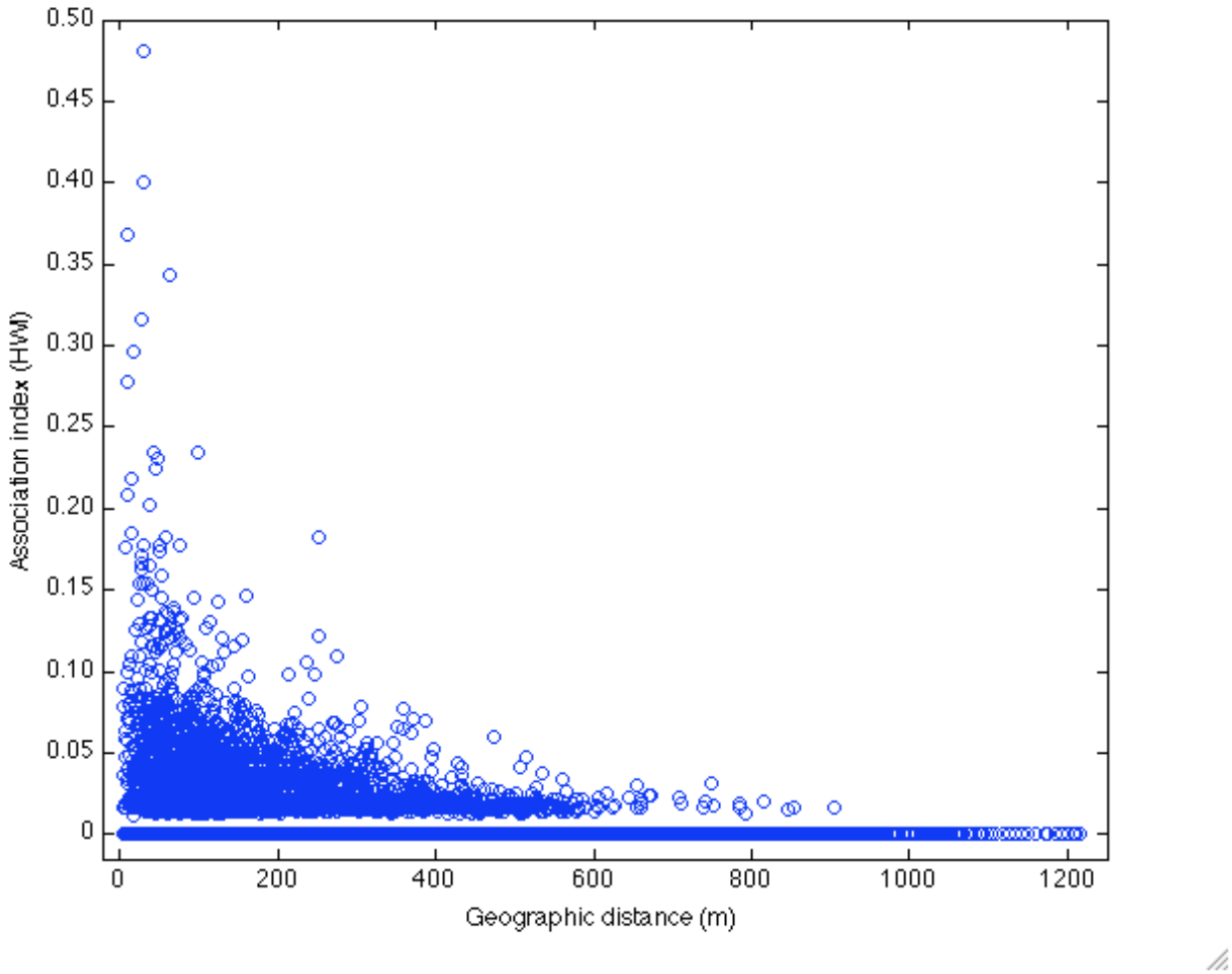


Figure 5. Relationship between pairwise relatedness and HWI for 76 adult female eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011 (n = 2,850).

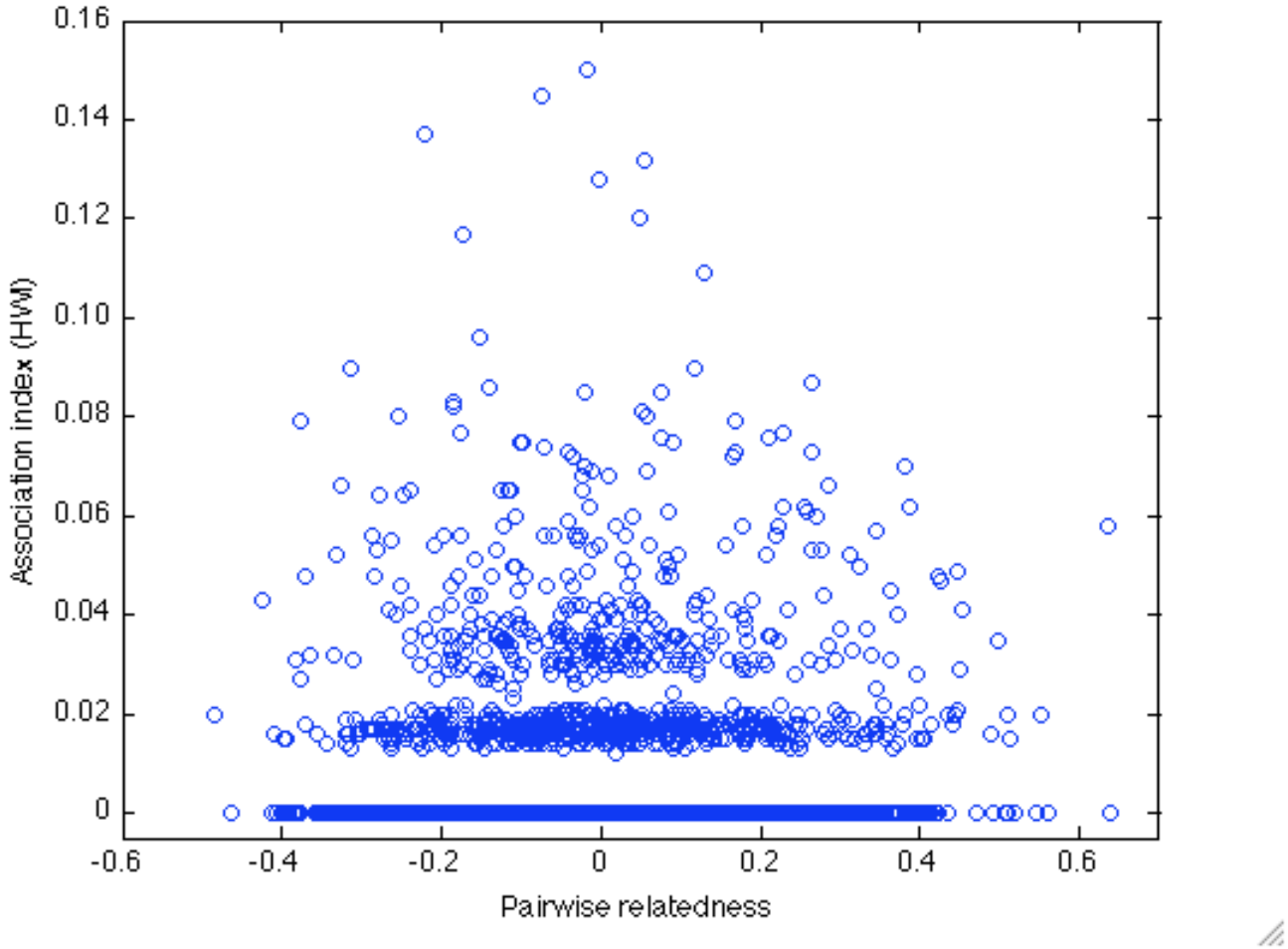


Figure 6. Mean proportion time spent alone (± 1SE) for 153 eastern grey kangaroos of different sex-age classes at Wilsons Promontory National Park, Australia, October 2010 to June 2011. Sex-age classes with the same letter above did not differ according to ANOVA and post-hoc Bonferroni multiple comparison tests ($P > 0.05$). AFY = adult female with a young-at-foot; AFN = adult female without a young-at-foot; AM = large adult male; SM = small male; SAF = sub-adult female; SAM = sub-adult male.

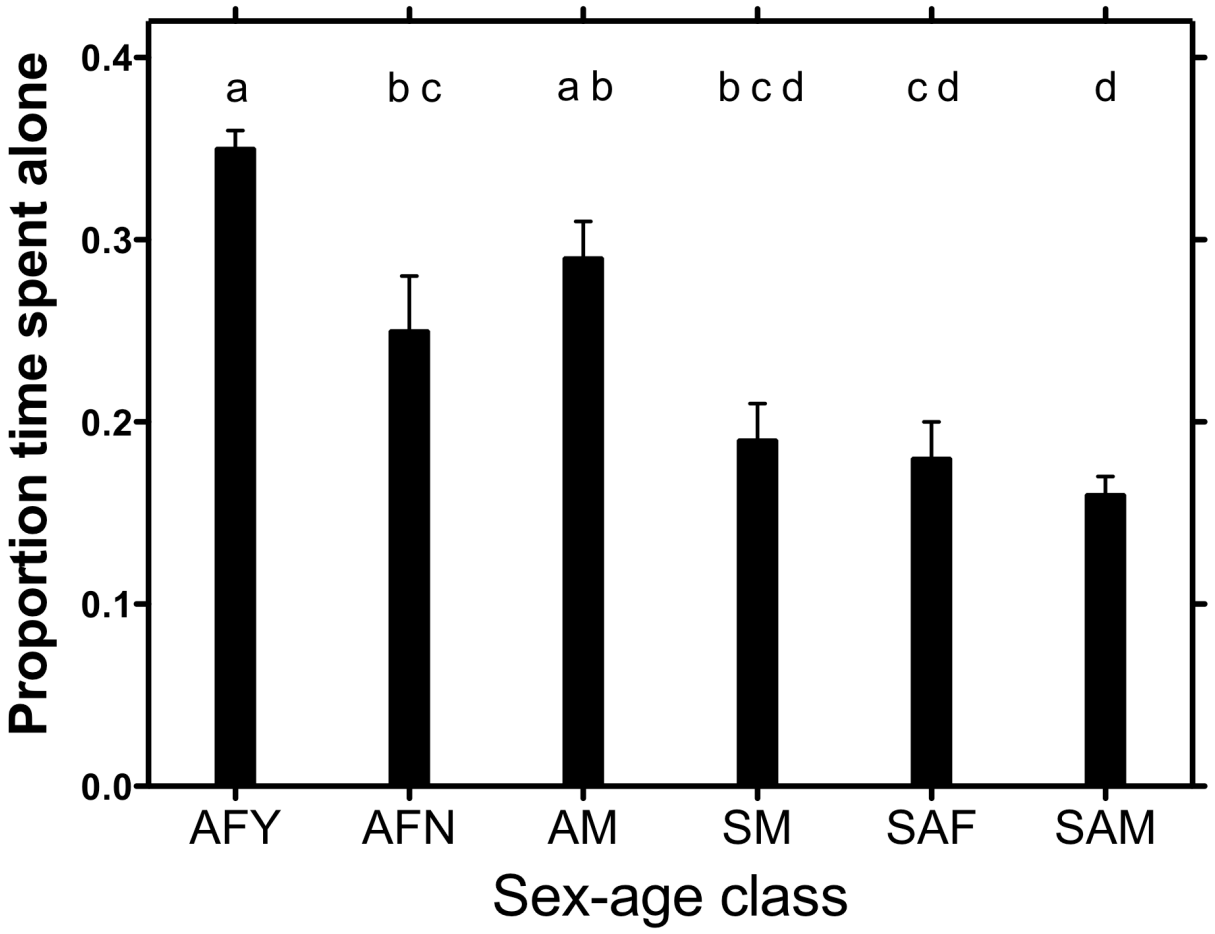


Figure 7. Relationship between sample size and mean HWI for different sex-age classes of 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011. AFY = adult female with a young-at-foot; AFN = adult female without a young-at-foot; AM = large adult male; SM = small male; SAF = sub-adult female; SAM = sub-adult male. The overall correlation is in black.

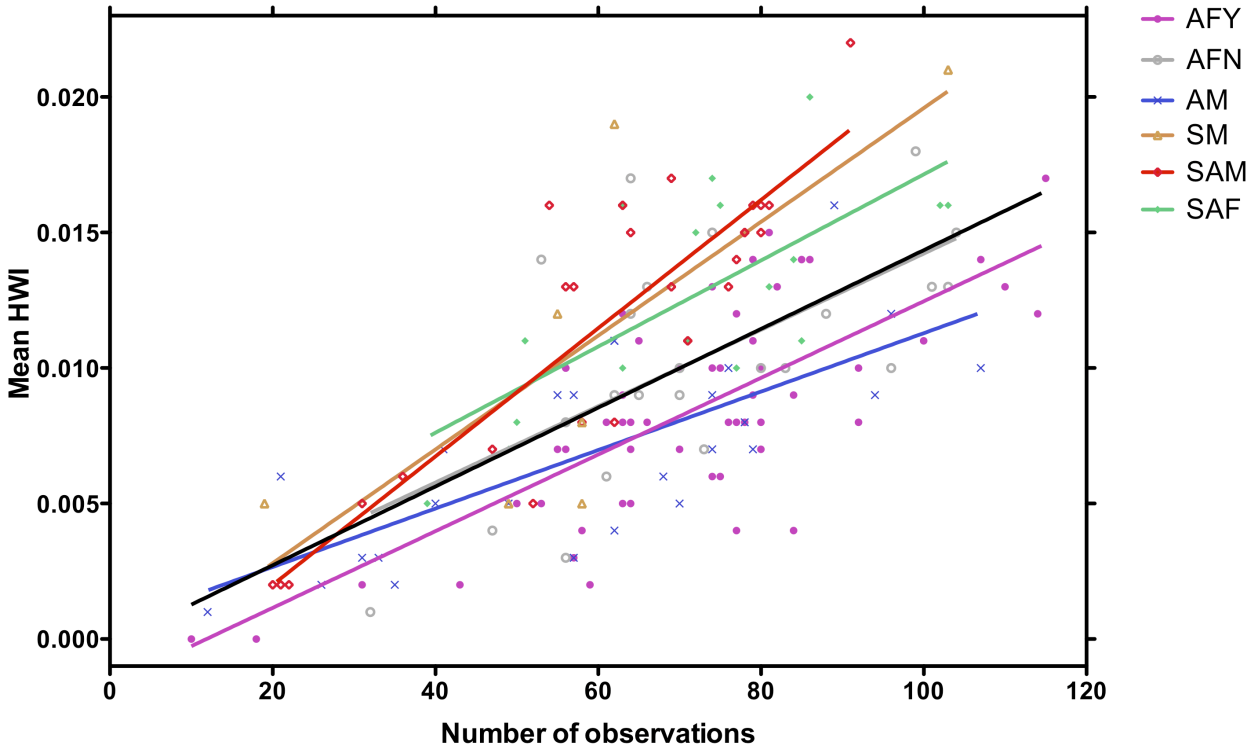


Figure 8. Relationship between sample size and number of associates for 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011.

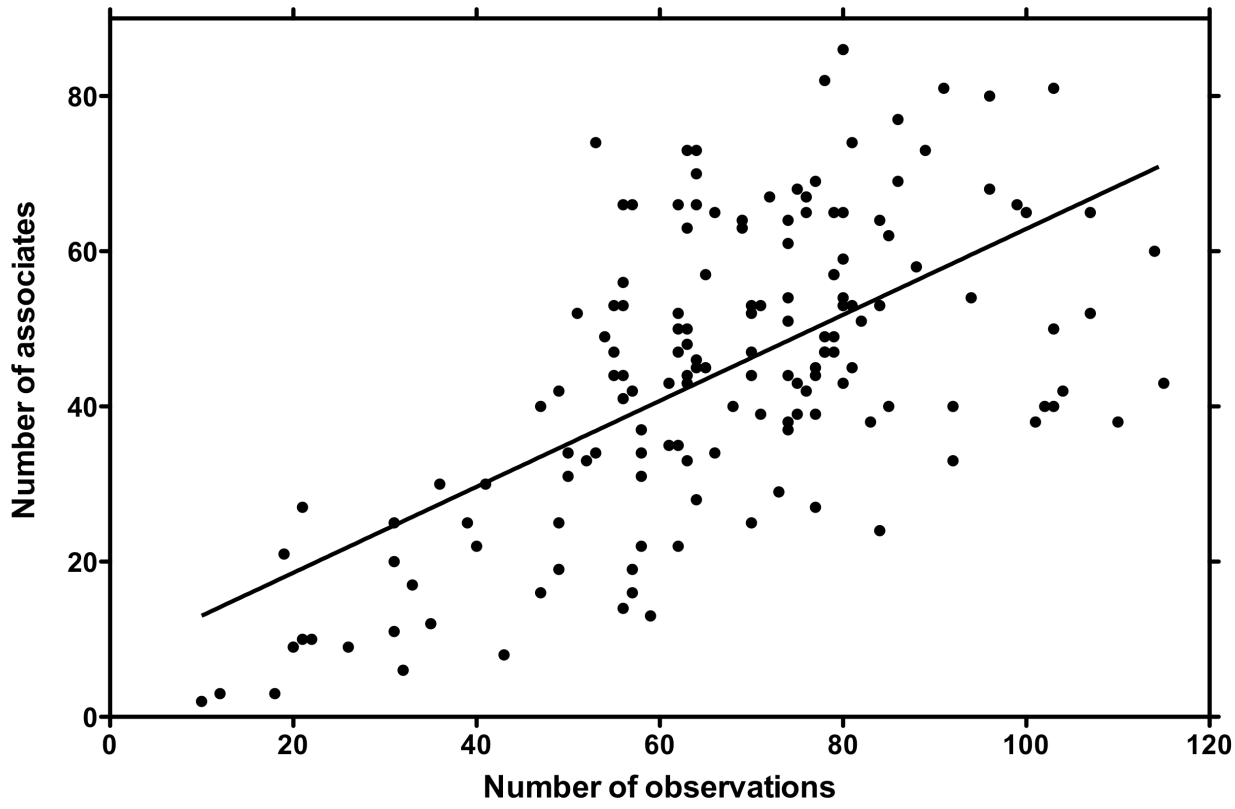


Figure 9. Relationships between sample size and reach (closed circles and solid line), strength (open circles and dashed line) and affinity (crosses and dotted line) for 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011.

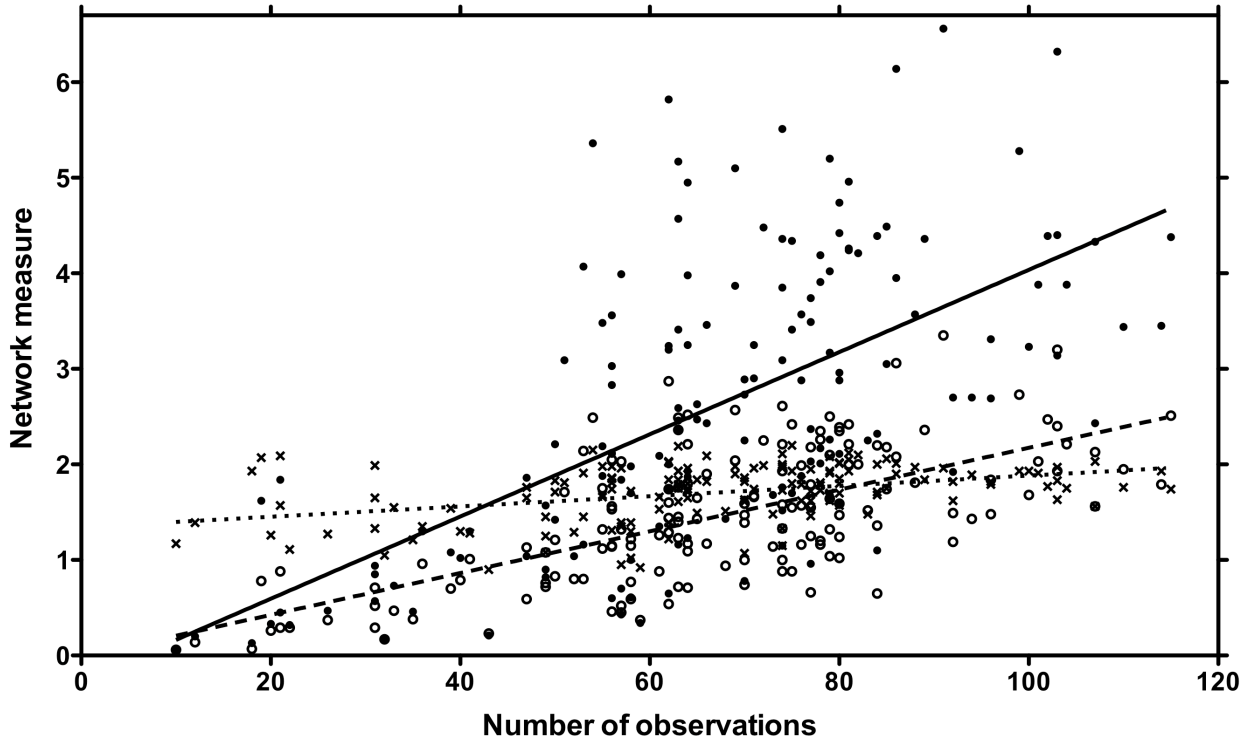
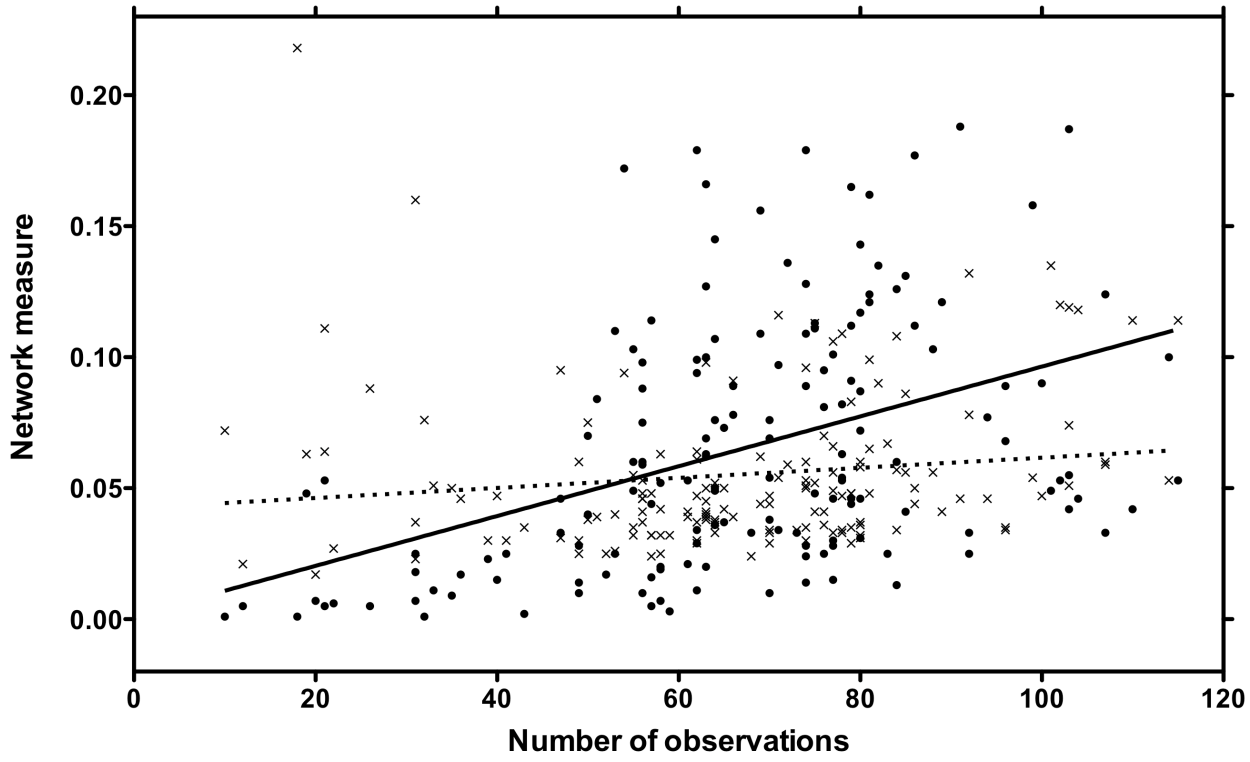
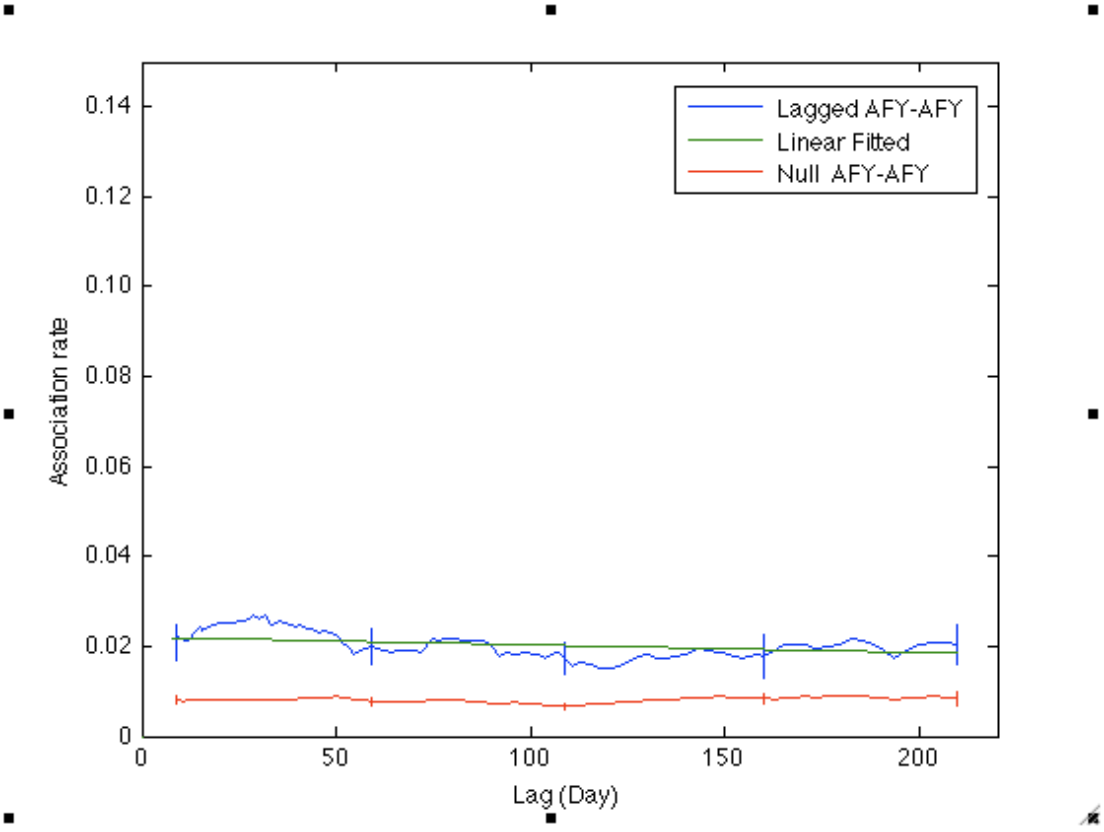


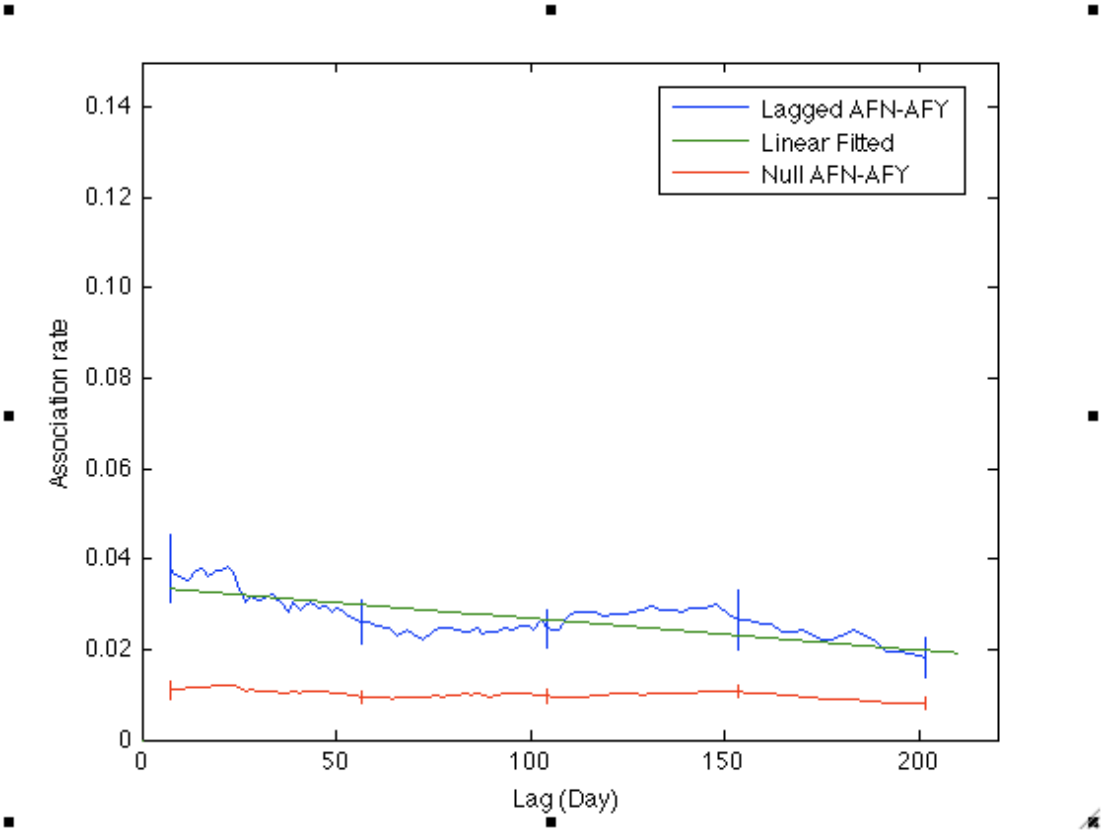
Figure 10. Relationships between sample size and eigenvector centrality (closed circles and solid line) and clustering coefficient (crosses and dotted line) for 153 eastern grey kangaroos at Wilsons Promontory National Park, Australia, October 2010 to June 2011.



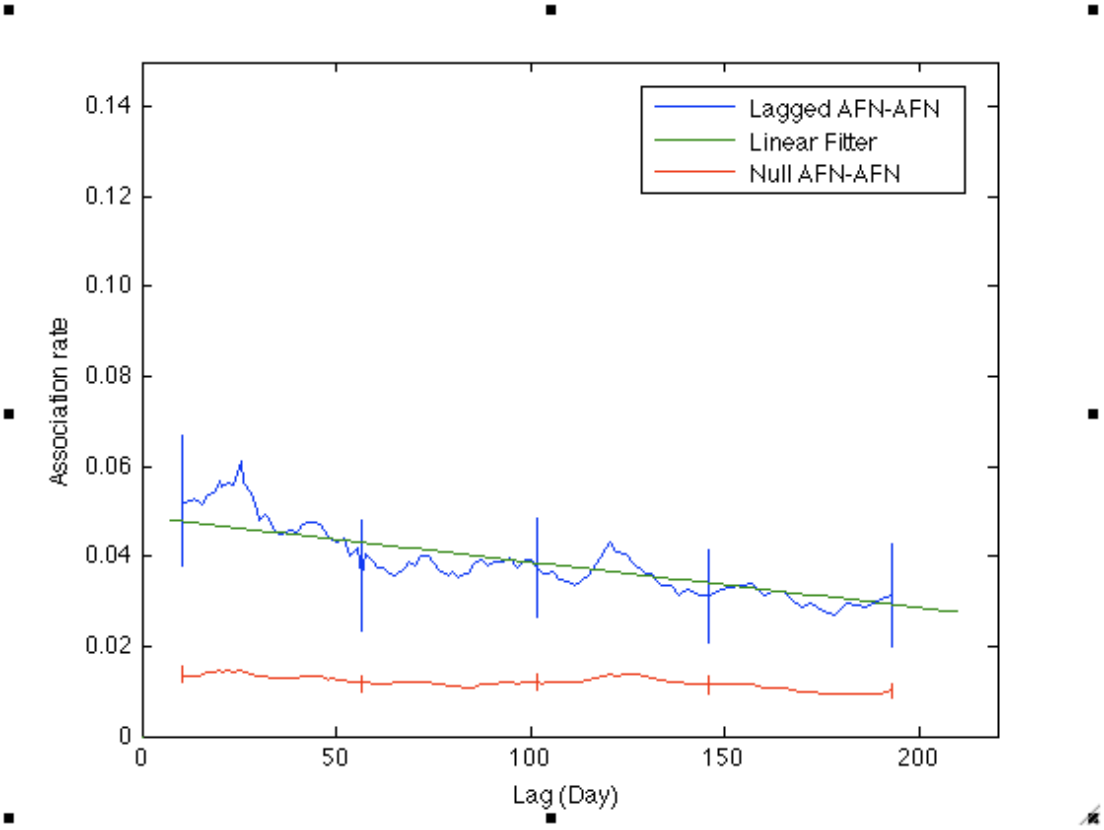
Supplementary Figure S1. Lagged association rates for AFY-AFY averaged over 7,000 associations.



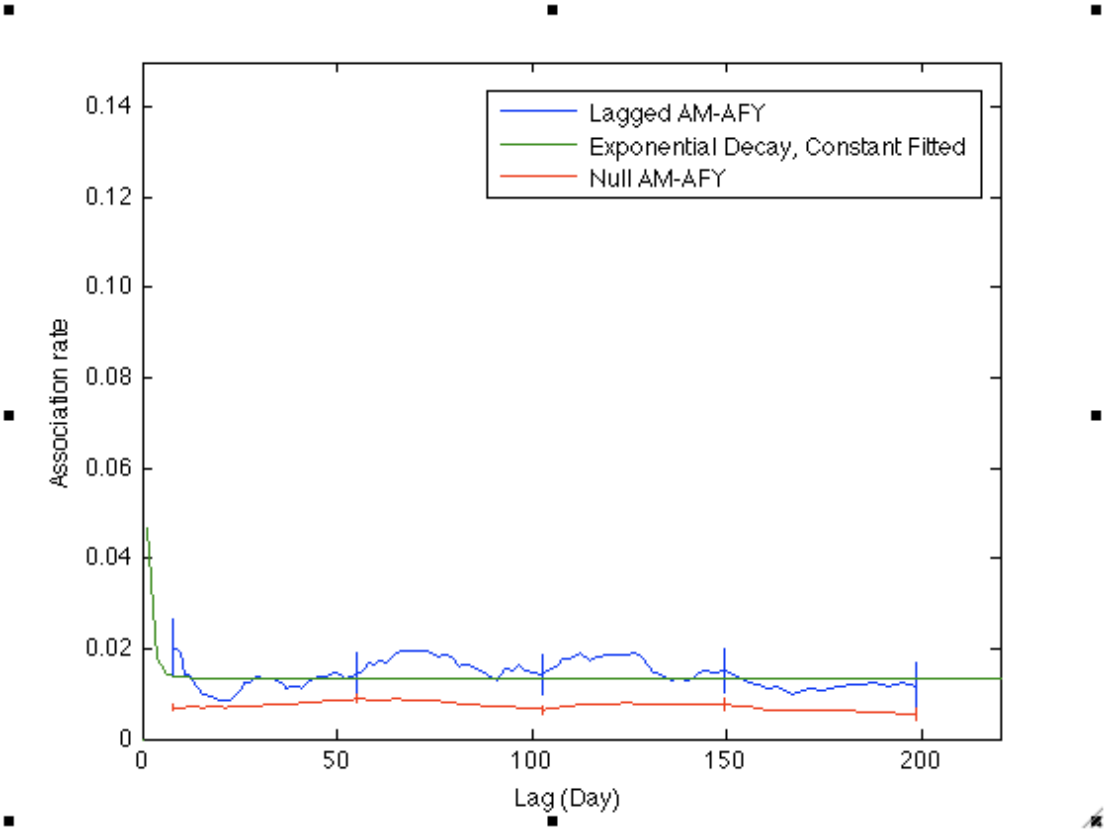
Supplementary Figure S2. Lagged association rates for AFN-AFY averaged over 3,000 associations.



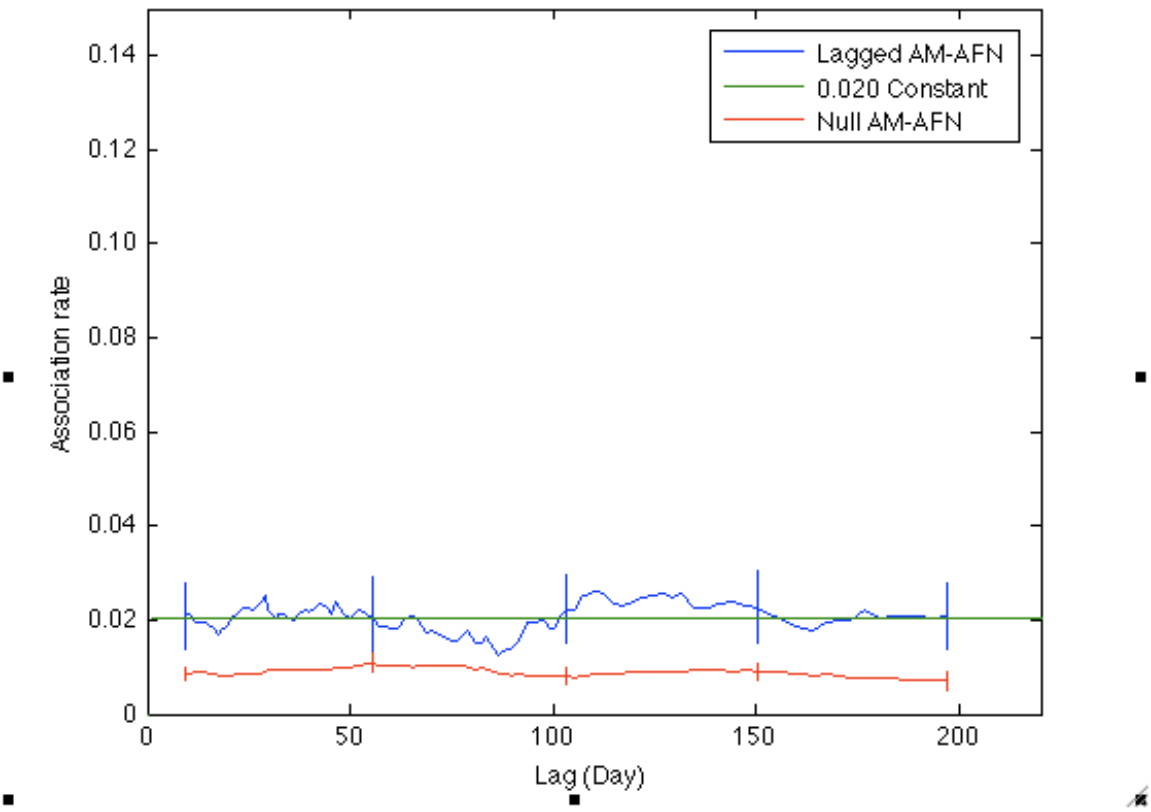
Supplementary Figure S3. Lagged association rates for AFN-AFN averaged over 2,000 associations.



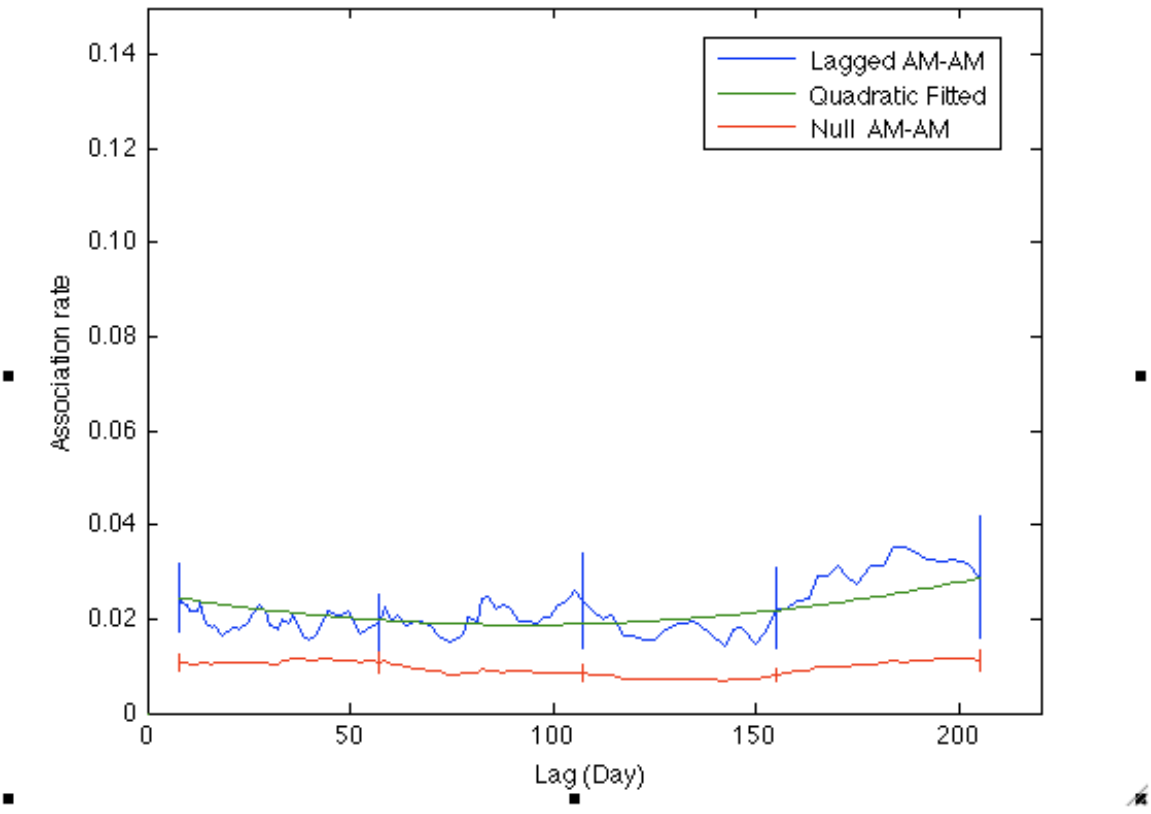
Supplementary Figure S4. Lagged association rates for AM-AFY averaged over 2,000 associations.



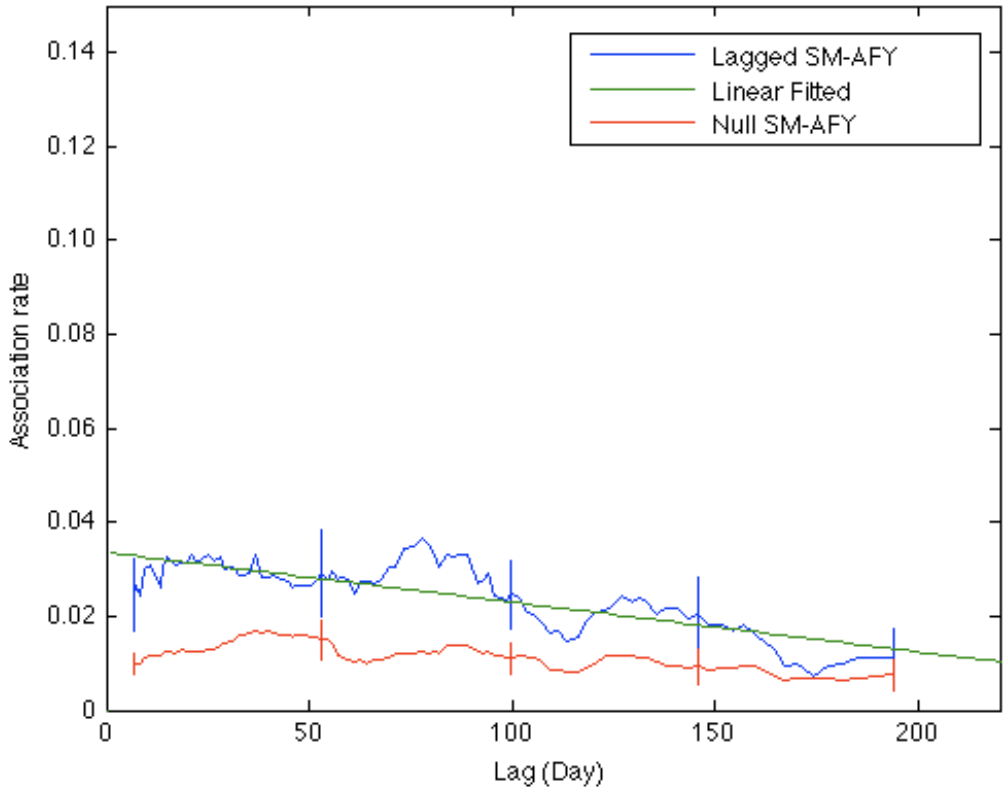
Supplementary Figure S5. Lagged association rates for AM-AFN averaged over 1,000 associations.



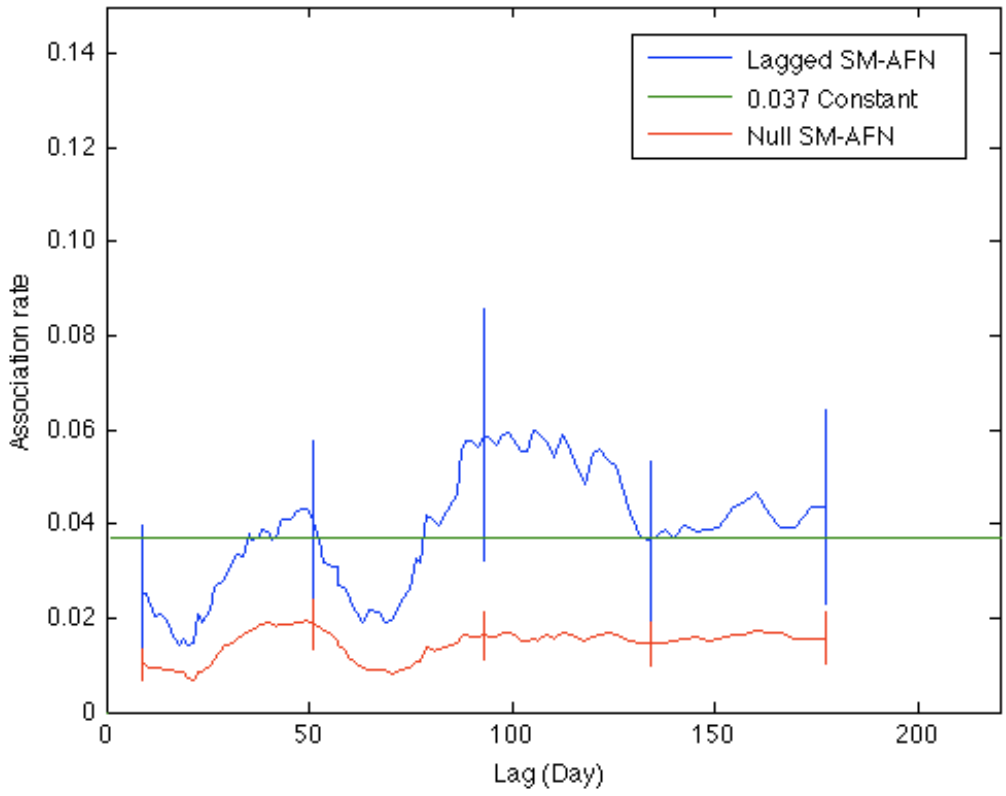
Supplementary Figure S6. Lagged association rates for AM-AM averaged over 1,500 associations.



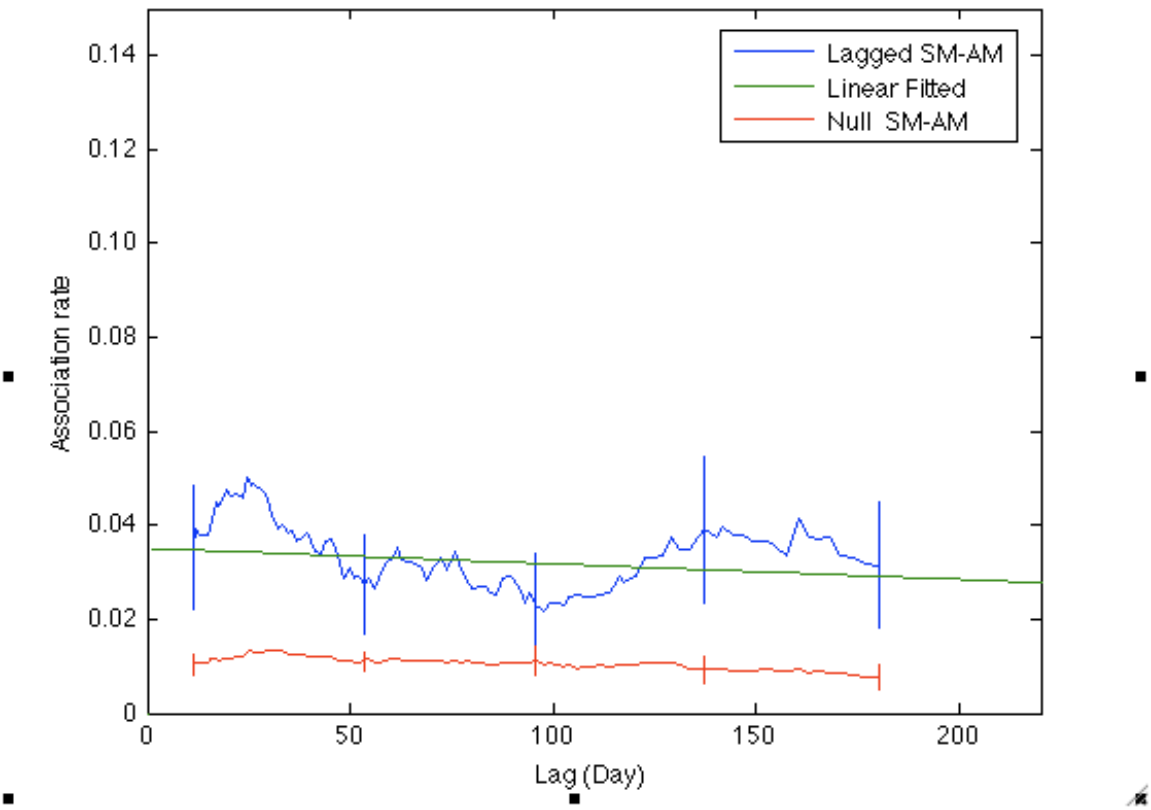
Supplementary Figure S7. Lagged association rates for SM-AFY averaged over 800 associations.



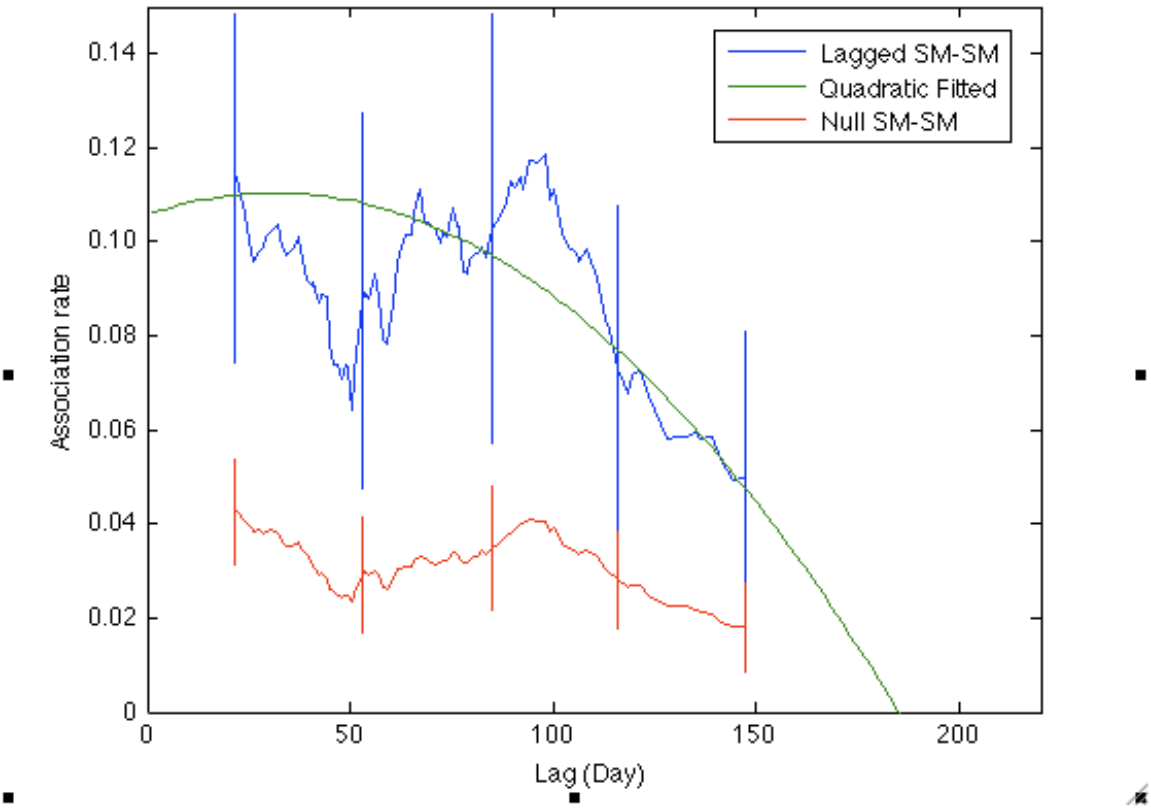
Supplementary Figure S8. Lagged association rates for SM-AFN averaged over 400 associations.



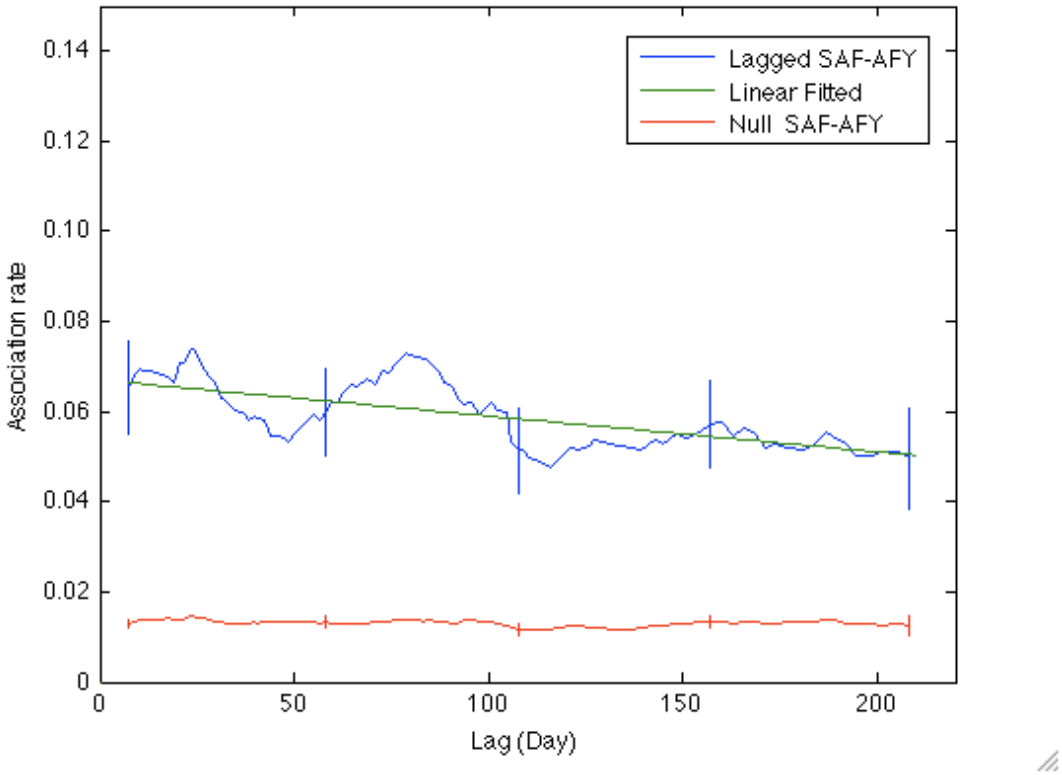
Supplementary Figure S9. Lagged association rates for SM-AM averaged over 500 associations.



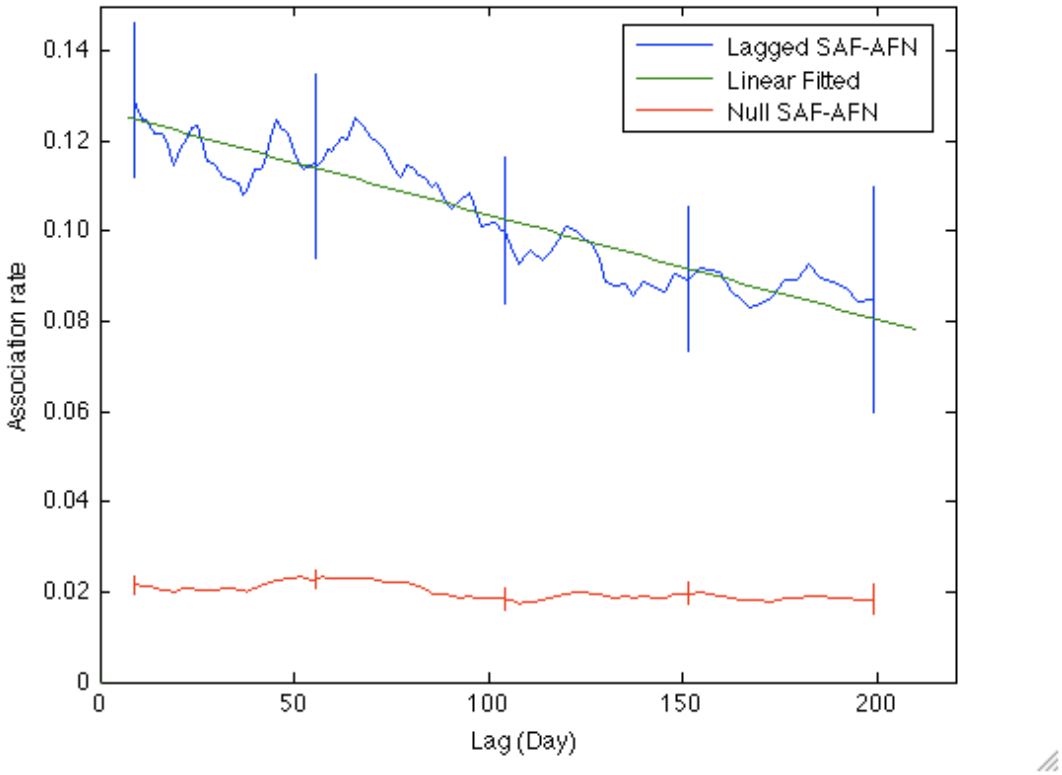
Supplementary Figure S10. Lagged association rates for SM-SM averaged over 400 associations.



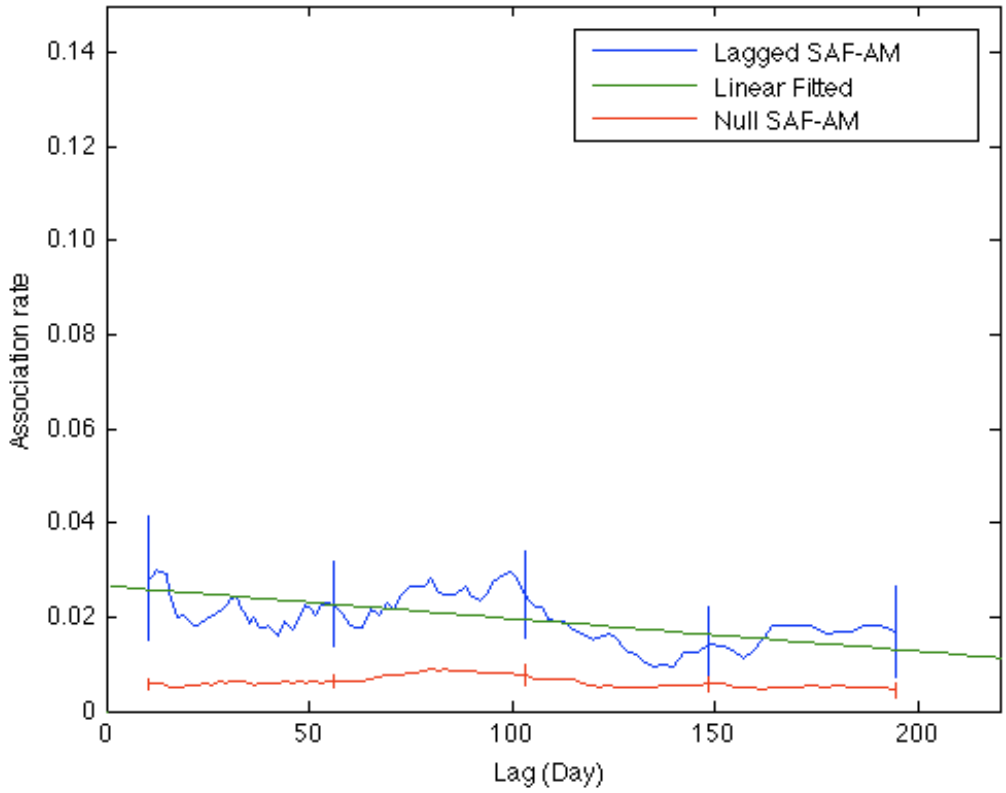
Supplementary Figure S11. Lagged association rates for SAF-AFY averaged over 3,000 associations.



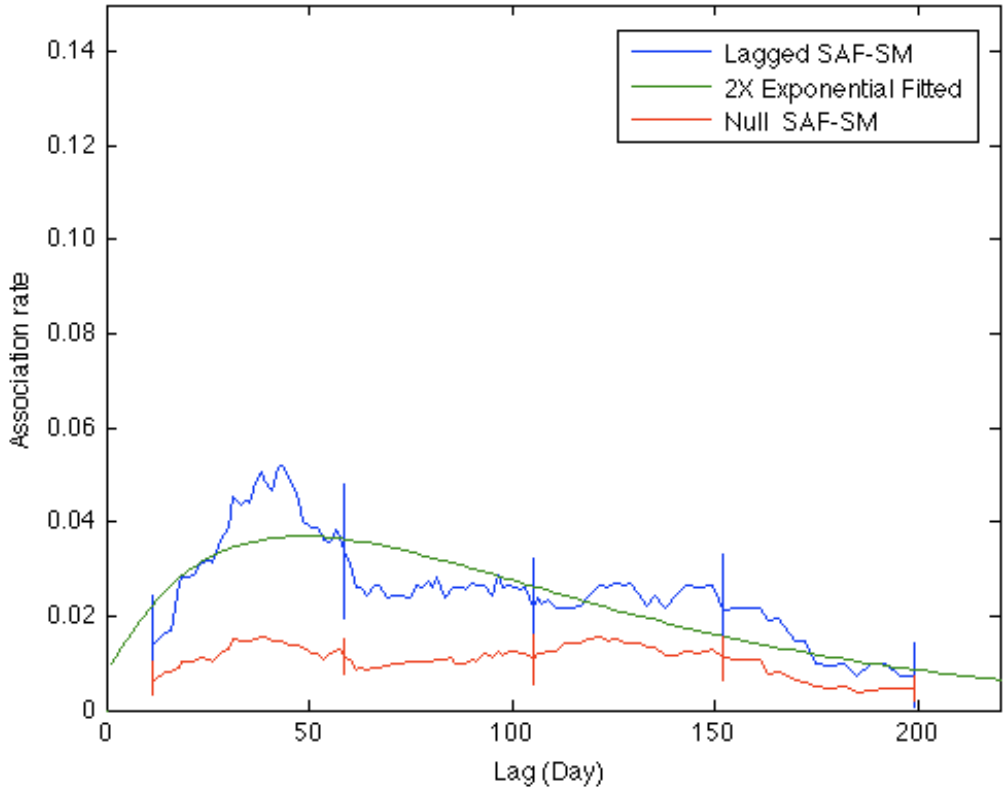
Supplementary Figure S12. Lagged association rates for SAF-AFN averaged over 2,000 associations.



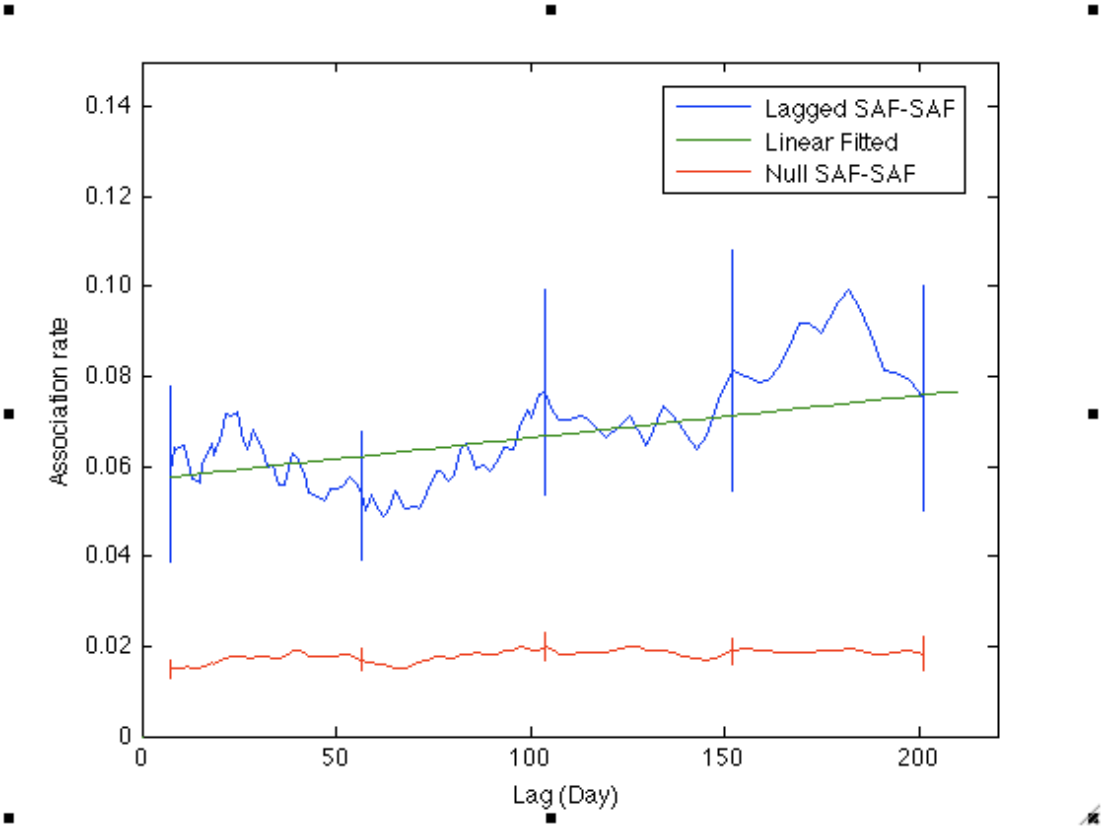
Supplementary Figure S13. Lagged association rates for SAF-AM averaged over 700 associations.



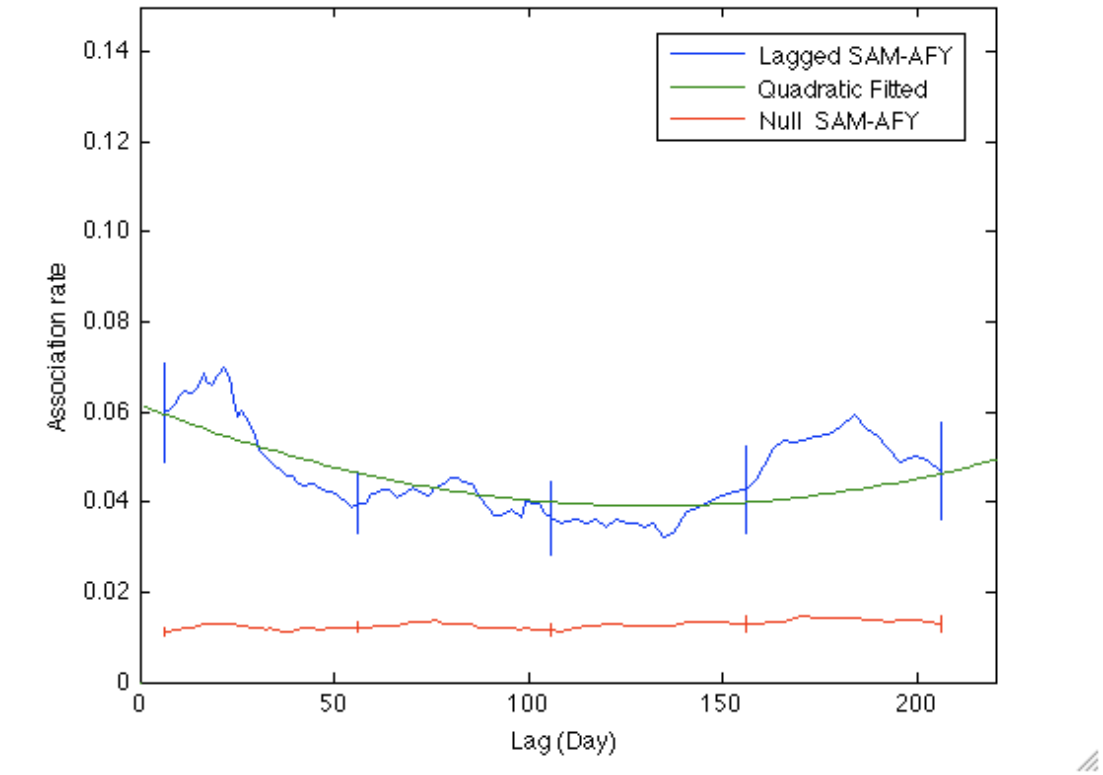
Supplementary Figure S14. Lagged association rates for SAF-SM averaged over 400 associations.



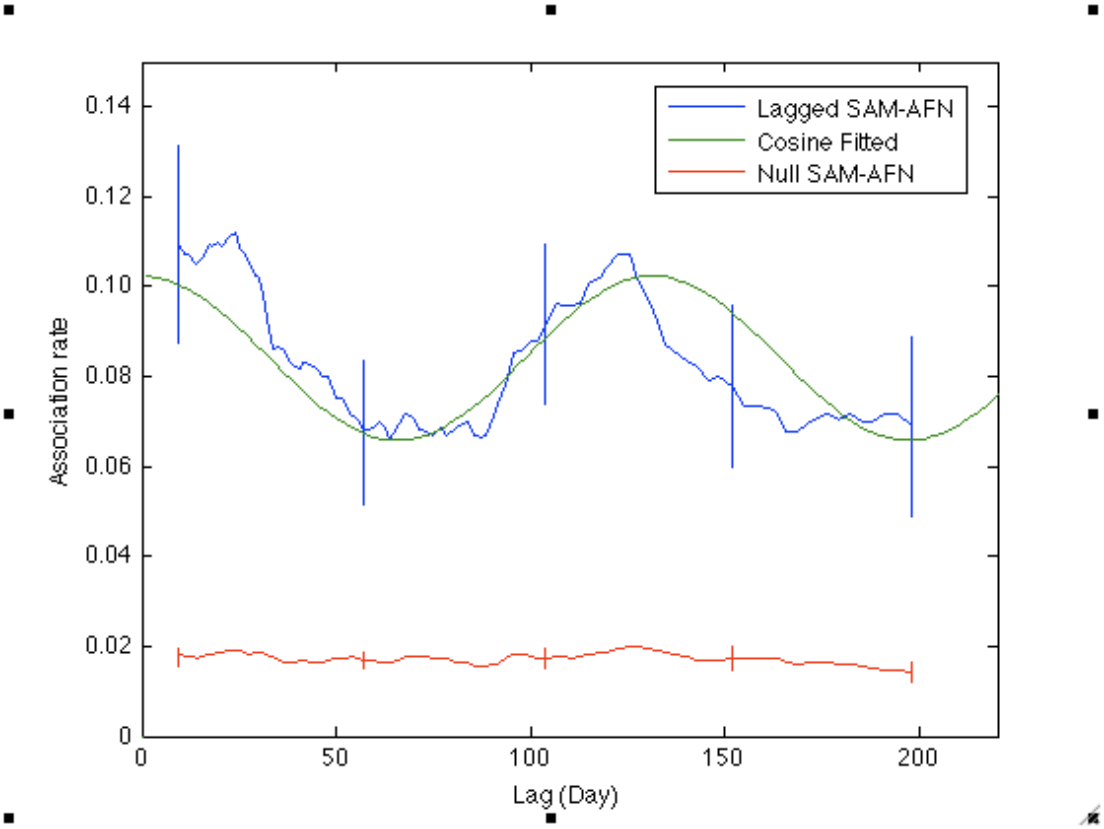
Supplementary Figure S15. Lagged association rates for SAF-SAF averaged over 1,200 associations.



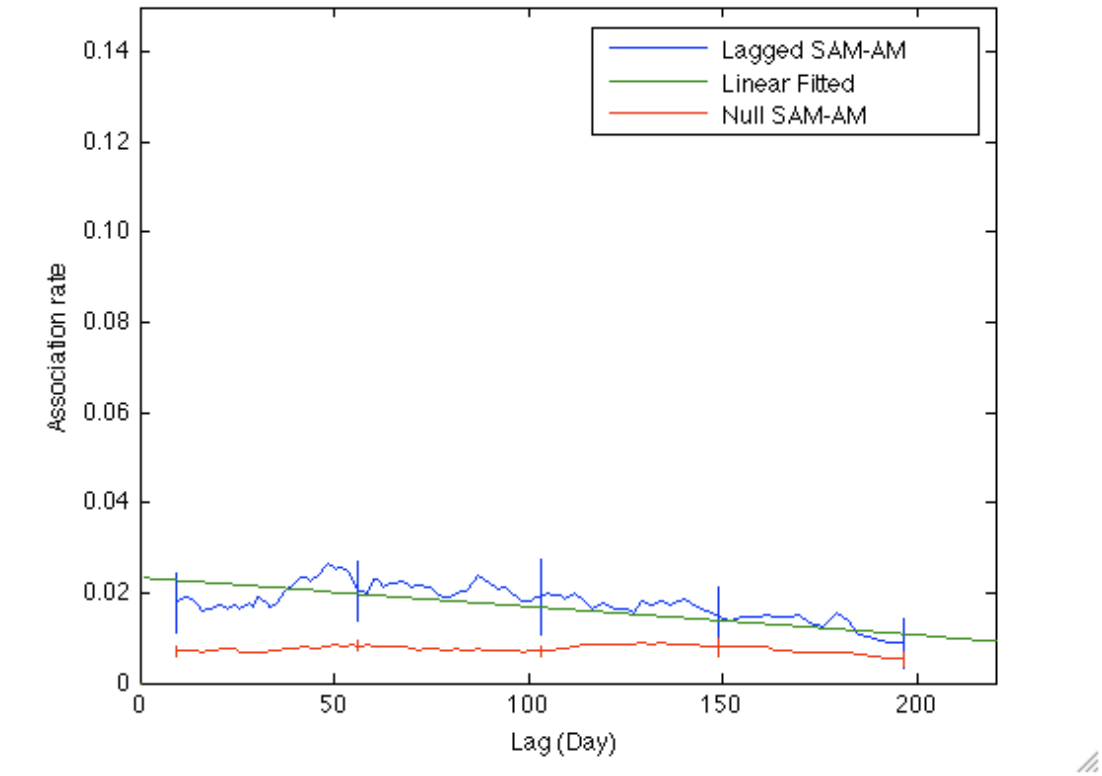
Supplementary Figure S16. Lagged association rates for SAM-AFY averaged over 3,000 associations.



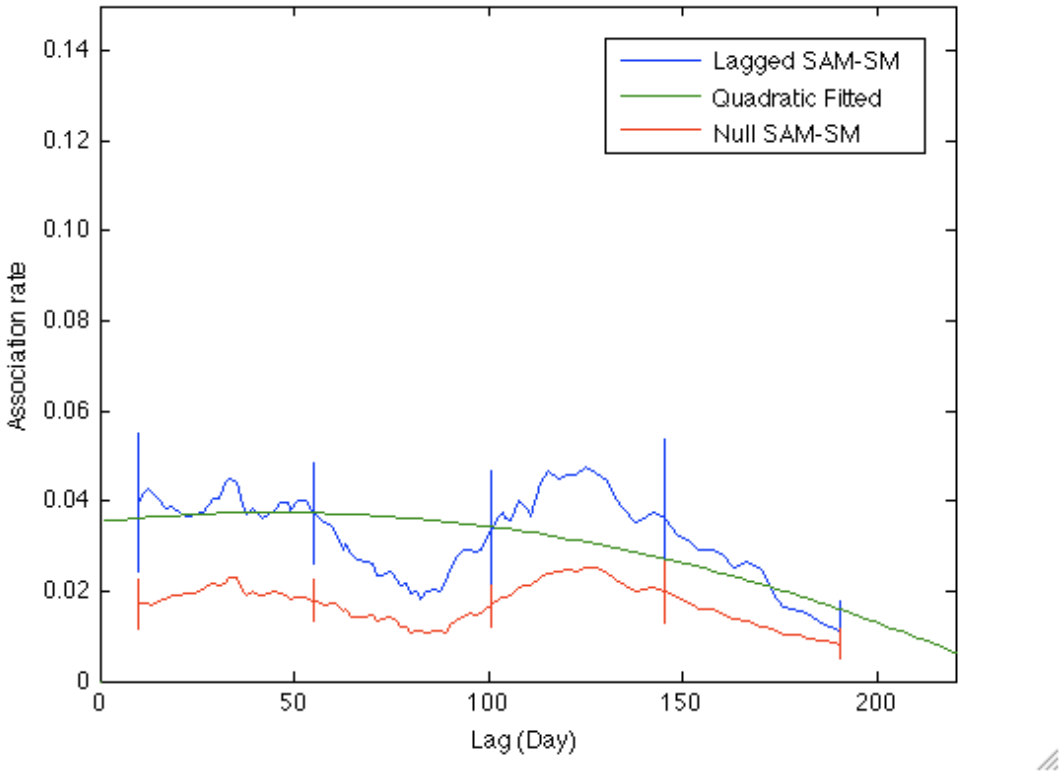
Supplementary Figure S17. Lagged association rates for SAM-AFN averaged over 2,000 associations.



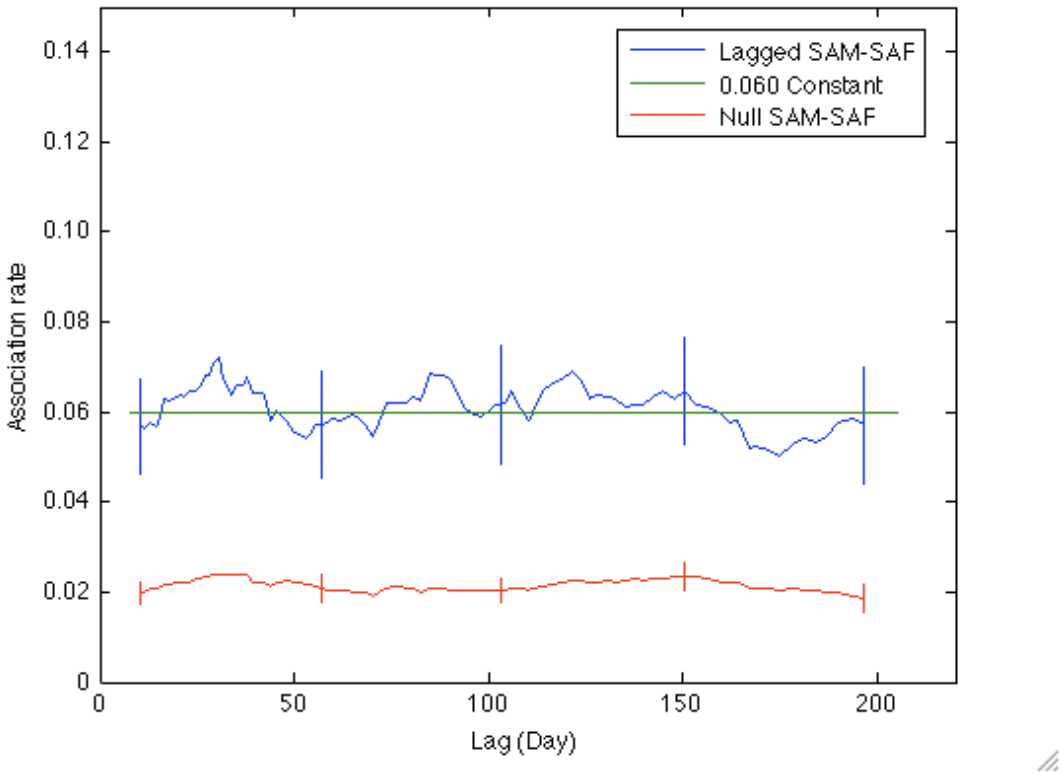
Supplementary Figure S18. Lagged association rates for SAM-AM averaged over 1,000 associations.



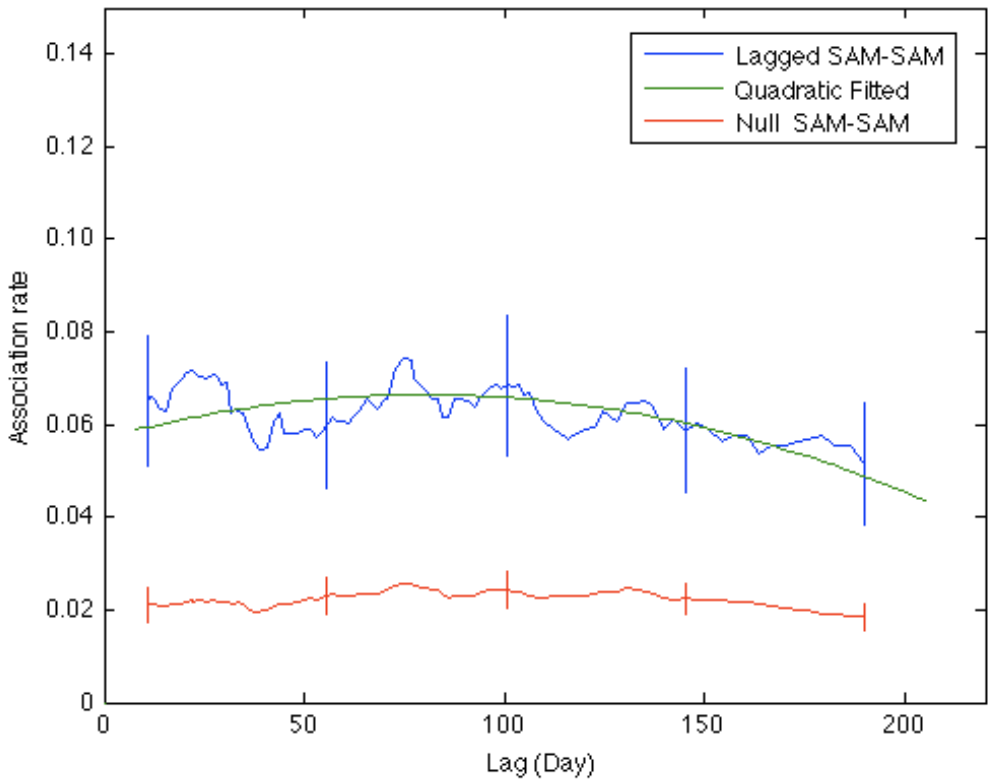
Supplementary Figure S19. Lagged association rates for SAM-SM averaged over 700 associations.



Supplementary Figure S20. Lagged association rates for SAM-SAF averaged over 2,000 associations.



Supplementary Figure S21. Lagged association rates for SAM-SAM averaged over 3,000 associations.



CHAPTER 3: Mother-offspring distances reflect sex differences in fine-scale genetic structure of eastern grey kangaroos

Wendy J. King, Dany Garant & Marco Festa-Bianchet
Manuscript published in *Ecology and Evolution* 5:2084-2094.

3.1 ABSTRACT

Natal dispersal affects life history and population biology and causes gene flow. In mammals, dispersal is usually male-biased so that females tend to be philopatric and surrounded by matrilineal kin, which may lead to preferential associations among female kin. Here we combine genetic analyses and behavioural observations to investigate spatial genetic structure and sex-biased dispersal patterns in a high-density population of mammals showing fission-fusion group dynamics. We studied eastern grey kangaroos (*Macropus giganteus*) over two years at Wilsons Promontory National Park, Australia, and found weak fine-scale genetic structure among adult females in both years but no structure among adult males. Immature male kangaroos moved away from their mothers at 18–25 months of age while immature females remained near their mothers until older. A higher proportion of male (34%) than female (6%) sub-adults and young adults were observed to disperse, although median distances of detected dispersals were similar for both sexes. Adult females had overlapping ranges that were far wider than the maximum extent of spatial genetic structure found. Female kangaroos, although weakly philopatric, mostly encounter non-relatives in fission-fusion groups at high density and therefore kinship is unlikely to strongly affect sociality.

3.2 INTRODUCTION

Natal dispersal is a fundamental parameter in life history and population biology and affects gene flow (Garant *et al.* 2007; Slatkin 1987). Sex-biased dispersal may be caused by inbreeding avoidance and/or benefits gained by one sex from increased access to resources or mates (Greenwood 1980; Pusey 1987), while factors promoting philopatry include benefits of familiarity with the natal area and kin cooperation (Lawson Handley & Perrin 2007). In mammals, dispersal is usually biased towards males, with females tending to be philopatric and thus surrounded by matrilineal kin (Greenwood 1980; Lawson Handley & Perrin 2007; Michener 1983) that may behave cooperatively (Hamilton 1964).

Fine-scale genetic structuring has been found among females in several species of mammals, *e.g*. black rhinoceros *Diceros bicornis* (Van Coeverden de Groot *et al.* 2011), white-tailed deer *Odocoileus virginianus* (Cullingham *et al.* 2011), red deer *Cervus elaphus* (Pérez-Espona *et al.* 2010), black bears *Ursus americanus* (Roy *et al.* 2012), bobcats *Lynx rufus* (Croteau *et al.* 2010) and Richardson's ground squirrels *Spermophilus richardsonii* (van Staaden *et al.* 1996). In contrast, genetic structuring is absent in other species, *e.g*. American badgers *Taxidea taxus* (Kierepka *et al.* 2012), Siberian lemmings *Lemmus sibericus* (Ehrich & Stenseth 2001), degu *Octogon degus* (Quirici *et al.* 2011) and inconsistent in rabbits *Oryctolagus cuniculus* (Richardson *et al.* 2002). Although allele frequencies provide information on average gene flow over many generations in the recent past, observed dispersal tendencies and distances provide complementary information on the extent and direction of movements at a particular time when environmental conditions may also be assessed (Lawson Handley & Perrin 2007; Slatkin 1987). Sex-biased dispersal appears to be an important catalyst of social evolution from multi-male multi-female aggregates to stable cooperative groups (Schultz *et al.* 2011). The extent of female philopatry in species showing flexible spatiotemporal grouping patterns ('fission-fusion' dynamics (Aureli *et al.* 2008)) is thus of interest.

Previous fine-scale studies of dispersal in large mammals in fission-fusion societies have focused on ungulates such as African savannah elephants *Loxodonta africana* (Archie *et al.* 2008), forest elephants *Loxodonta cyclotis* (Schuttler *et al.* 2014), wild boar *Sus scrofa* (Poteaux *et al.* 2009), red deer (Nussey *et al.* 2005), white-tailed deer (Mathews & Porter 1993) and domestic sheep *Ovis aries* (Coltman *et al.* 2003; Nituch *et al.* 2008). In all these species, females and males follow the typical mammalian male-biased dispersal pattern with persistent kin associations among adult females.

Little is known about dispersal in marsupials. Asocial common wombats *Vombatus ursinus* and semi-social southern hairy-nosed wombats *Lasiorhinus latifrons* are unusual among mammals in showing female-biased dispersal, which may occur post-breeding (Banks *et al.* 2002; Walker *et al.* 2008). Both sexes can be philopatric in communally nesting agile antechinus *Antechinus agilis* (Banks *et al.* 2005). Females, but not males, are philopatric in semi-social mountain brushtail possums *Trichosurus cunninghami* (Blyton *et al.* 2014). Brush-tailed rock wallaby *Petrogale penicillata* females show strong philopatry (Hazlitt *et al.* 2004) in a social system with discrete multi-male multi-female groups (Laws & Goldizen 2003). To date, however, there have been no detailed studies of dispersal in marsupials that live in fission-fusion groups.

Eastern grey kangaroos *Macropus giganteus* are large marsupials that can form extensive aggregations in open habitat (Jaremovic & Croft 1991). The social structure is fission-fusion, with individuals frequently joining and leaving groups that typically range in size between 3 and 10

individuals (Jarman & Coulson 1989). Only weak genetic structuring has been found at scales over 20 km, although range-wide genetic analyses showed that more dispersers are male than female (Zenger *et al.* 2003). Females are generally thought to be sedentary but long-range movements (>12 km) post-breeding have been documented (Coulson *et al.* 2014; Jarman & Taylor 1983). A recent study suggested that females were highly philopatric at a fine scale and exhibited preferential behaviour towards kin (Best *et al.* 2014; Best *et al.* 2013). That study, however, did not examine patterns in males, pooled 2 years of observations and did not distinguish adults from sub-adults, therefore sub-adult females were likely sampled while they were still closely associated with their mother. It is thus unclear to what extent adult females are philopatric and show positive genetic structuring at a fine scale in this species showing fission-fusion dynamics. Our aim was to determine the role of dispersal and settlement patterns in the genetic structure of both males and females in a population of eastern grey kangaroos at high density.

We first described the fine-scale genetic structure of eastern grey kangaroos for adults of both sexes using spatial autocorrelation analyses. To contrast observational data to genetic structure, we examined distances between mothers and offspring as the latter aged from permanent pouch emergence to sexual maturity and beyond. In addition, we compared dispersal tendencies of young males to those of young females. Finally, we interpreted results from spatial autocorrelation analyses in light of individual range sizes.

3.3 MATERIALS AND METHODS

We studied eastern grey kangaroos at Wilsons Promontory National Park, Australia (38° 57' S, 146° 17' E) from April 2010 to June 2012, as part of a long-term monitoring program that started in 2008. Kangaroos inhabit a 110-ha study area that consists of meadows surrounding a grassy landing strip. The area is mostly open with occasional trees and bushes such as coast tea-tree *Leptospermum laevigatum*, coast wattle *Acacia longifolia* and coast banksia *Banksia integrifolia* (Davis *et al*. 2008). Densities of kangaroos were high (approximately 6 individuals/ha) both years (Glass 2013). We captured and marked about 50% of adult females and 80% of adult males following King *et al.* (2011). Animals were aged at first capture according to their mass and reproductive status (presence of pouch young or extended teats in females). Adult females weighed 20–35 kg and adult males 36–63.5 kg. We determined mother-offspring relationships by capturing young in the pouch or as suckling young-at-foot. Known adult mother-daughter pairs had been monitored from when the daughters were caught as pouch young and genetic relationships were confirmed using pair-wise relatedness coefficients (see below). We estimated birthdates of pouch young based on hind leg, hind foot and head lengths according to Poole *et al*. (1982). This species

reaches sexual maturity in captivity at 2 years in females and 4 years in males (Poole & Catling 1974). Only one of 30 females first caught as pouch young reproduced before 3 years of age; that female was classed as an adult but other 2-year-old females were not. Sub-adults were mostly 22–30 months old (2-year-olds) in October through June and young-at-foot were 10–18 months old (1-year-olds). For some analyses of observational data, we separated males into a small male category (25–36 kg; 3- and 4-year-olds) and large adult males (>38 kg; at least 5 years old).

A small tissue sample (approximately 2 mm diameter) was collected from the ear of each individual at first capture, preserved in 95% ethanol and refrigerated at 4ºC until laboratory analyses. DNA extractions were carried out using the salting-out protocol described in Chambers & Garant (2010). DNA concentration was initially determined for each sample by gel electrophoresis and diluted to a final concentration of 5ng/ml for polymerase chain reaction (PCR) amplification.

Microsatellite amplification was performed at 9 loci using a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA). Multiplex PCR conditions and reaction mixture recipes are provided in Tables S1 and S2. PCR products were visualised using an AB 3130x capillary DNA sequencer (Applied Biosystems) by adding 0.15 ml GeneScan 600 LIZ (Applied Biosystems) internal size standard and 8.35 ml Hi-Di Formamide (Applied Biosystems) to 1.5 ml total PCR products. Allele size was assessed using GeneMapper version 4.1 (Applied Biosystems).

We used KINGROUP v2 (Konovalov *et al.* 2004) to calculate observed and expected heterozygosity and deviation from Hardy-Weinberg equilibrium at each locus. Pair-wise relatedness coefficients (*r*) were estimated in KINGROUP as per KINSHIP (Goodnight & Queller 1999; Queller & Goodnight 1989). To check for independence of loci, we tested for linkage disequilibrium using log likelihood ratios in GENEPOP 4.2 (Raymond & Rousset 1995; Rousset 2008).

Observations of marked individuals took place using 8X32 binoculars (Leica, Wetzlar, Germany) for 9-15 days per month from April 2010 to March 2012, occurring while most individuals were feeding for about 2.5 hours after dawn and before dusk. Additional observations occurred on 5 days per month from May to June 2012. Individuals' locations were recorded on foot from a distance of approximately 15 m using a hand-held Global Positioning System unit (GPSmap 60Cx, Garmin, Olathe, KS) with a precision of 4 m, and adjusted for observer/animal distance using a range-finder (SCOUT1000, Bushnell, Lenexa, KS) and compass (KB-14/360R, Suunto, Keili, Finland). Only the first location of an individual each day was used, to avoid temporal autocorrelation (Swihart & Slade 1985, 1997). Observations of adults were divided into two years: 15 April 2010 to 16 March 2011 and 5 April 2011 to 10 March 2012.

We used locations of individuals during foraging periods to calculate foraging range and core area sizes as 95% and 50% fixed kernels (Worton 1989). We employed Ranges8 version 2.5 (Kenward *et al.* 2008) and a smoothing factor *h* of 0.63, which was obtained as the median using least-squares cross validation (Kenward 2001), and a minimum of 30 locations per individual (Seaman et al. 1999). The size of cumulative 95% kernels approached an asymptote at 25 locations. Very occasionally, individuals made excursions from their foraging range (defined as being seen at a location outside the 110-ha area that was at least 500 m from any other location for that individual, usually along a road or track) and these sightings were excluded from analyses (12 of 6,876 sightings involving 6 adults in 2010–2011 and 15 of 8,043 sightings involving 6 adults in 2011–2012), including the range size calculations. A few sightings by Park staff were verified to establish individual identities. Because we only observed kangaroos while they were foraging, we possibly underestimated their home range sizes; however, kangaroos usually rested in the same areas as where they foraged and our observations included the 3-hour period after dawn, when kangaroo movements are greatest (Clarke *et al.* 1989). More adult females (72% of 106 individuals) than males (50% of 50 individuals) observed in the first year were also observed in the second year. Adult females were seen on average 62.4 ± 2.6 times in 2010–2011 and 57.8 ± 2.4 times in 2011–2012; adult males were seen 44.8 ± 4.0 times in 2010–2011 and 38.0 ± 2.9 times in 2011–2012. Many adult males were seen only 10–29 times per year (15/39 individuals in 2010–2011 and 19/50 individuals in 2011–2012) and so we did not use kernel analysis to estimate centres of activity, in order to maximise the number of individuals.

We calculated centroids as mean *x, y* coordinates for adults seen at least 10 times on the study area in either year. Next, we calculated centroids for adults seen both years as the overall mean *x, y* coordinates if seen at least 10 times in either year. Similarly, we calculated centroids for mothers and their offspring seen at least 10 times during 4-month periods when offspring were 10 to 41 months of age. Individuals were seen 20.6 ± 0.3 times per 4-month period.

We used the program GenAlEx 6.5 (Peakall & Smouse 2006, 2012) to calculate matrices of pair-wise genetic distances among adults according to Peakall *et al.* (1995) and Smouse & Peakall (1999). Genetic distance matrices for each locus were summed across all loci under the assumption of independence to generate a total genetic distance matrix. We then constructed a geographic distance matrix consisting of the Euclidean distance (in metres) between all pairs of centroids. To analyse global autocorrelation, the total genetic distance matrix was compared to the geographic distance matrix using a Mantel (1967) test and 9,999 random permutations. Analyses were first conducted on the overall dataset and then for each sex and year separately.

To investigate local autocorrelation, we correlated genetic distances with geographic distances at increasing distance classes of 15, 20, 25, 50, 75 and 100 m in GenAlEx 6.5 using 9,999

permutations to determine confidence intervals and 10,000 bootstrap resampling for standard errors, and plotted the resulting correlograms. We chose these distance classes based on pair-wise sample sizes and a previous study that found positive genetic structure up to 80 m among females (Best *et al.* 2014). Previous studies have often used relatedness coefficients rather than genetic distance to describe genetic structure of mammalian populations (Lawson Handley & Perrin 2007), thus we also briefly describe the relationship between relatedness and geographic distance when a positive genetic structure was detected in the initial analysis.

To assess factors affecting distances between centroids of mothers and known offspring, we used linear mixed-effects models in the R environment version 2.15.2 (R Development Core Team 2012) with offspring identity included as a random factor. Mother identity was also initially included as a random factor in the model but was not significant. Offspring age (linear and measured in 4-month periods), offspring sex (factor) and year (factor) were included as fixed effects in models. Distance between centroids was first transformed using $\log(x+1)$ to assure normal distribution of model residuals. We sequentially removed the least significant parameter (based on its *P*-value, threshold ≥ 0.05) from the model using stepwise backward selection (Crawley 2007). Differences between logarithmically transformed range sizes of different age-sex classes were assessed using ANOVA and Bonferroni *post-hoc* tests.

We used *t*-tests to compare the distances over which adult males and females moved their centroids from one year to the next. Distances moved were transformed (log) to ensure normal distribution and results are presented back-transformed. Proportions of males and females that dispersed (born on the study area and seen at least 750 m from their original place of capture) were compared using Fisher Exact tests and calculated for 3 cohorts (birthdates September–May 2006/2007, 2007/2008 and 2008/2009) monitored from August 2008 to August 2014. Dispersing individuals were opportunistically sighted along roads and tracks. We chose 750 m as the minimum distance for dispersal because, as noted above, individuals were rarely seen more than 500 m from another of their own locations and no sub-adults or small males were seen at distances between 574 and 810 m from their original place of capture. Because we did not always have sufficient observations to calculate centroids of dispersing individuals, we used the original place of capture, which was as a pouch young on 70% of occasions, to measure dispersal distances. We employed Mann-Whitney *U*-tests to compare distances between centroids of mothers and known sons or daughters at different ages and to compare dispersal distances of males *vs.* females. Because the distance between known offspring and their mothers did not appear to increase linearly as they aged, we fitted quadratic lines to the data and compared the fit to linear models using extra sum-of-squares *F*-tests. We then chose the non-linear model if it differed significantly ($P < 0.05$) from the linear model. We used Pearson correlations to compare distances between centroids of adults and

their pair-wise relatedness where genetic structure had been found and sample sizes were most robust, *i.e.*, for adult females within 25 m.

Spreadsheets of data have been archived at Dryad Digital Depository http://dx.doi.org/10.5061/dryad.b999q, including 1) genotypes and mean *x, y* coordinates for adult kangaroos by sex each year, 2) distances between mothers and young of different ages and sexes, 3) range sizes of 95% and 50% kernels for different sex-age classes and 4) known dispersers with sex, age and distance moved.

3.4 RESULTS

3.4.1 Microsatellite Analyses

The 9 microsatellite loci were polymorphic and did not deviate from Hardy-Weinberg equilibrium (Table S3). Linkage disequilibrium occurred in 1 out of 36 possible combinations of loci (2.8% of combinations; loci G26-4 and T3-1T; P <0.001) and therefore spatial genetic autocorrelation analyses were repeated excluding G26-4. Results were quantitatively similar and thus we present analyses using the 84 alleles across all 9 loci (Table S3).

3.4.2 Spatial Autocorrelation

Global spatial autocorrelation analyses revealed a weak positive spatial structure for females in both years (2010–2011, r_{xy} =0.077, P = 0.036 and 2011–2012, r_{xy} =0.095, P = 0.008; Table 1). In contrast, males showed no genetic structure in either year (2010–2011, r_{xy} = 0.028, P = 0.31 and 2011–2012, r_{xy} = 0.035, P = 0.25; Table 1). Maximum distances were 971 and 1,363 m between male centroids and 1,204 and 1,337 m between female centroids for the two years, respectively. Using distance classes of 15, 20 and 25 m, local autocorrelation analyses showed significant spatial structure for females in the first year at the 0–15, 0–20 and 0–25 m classes and random beyond about 50 m (Table 2). There were also weak positive correlations at 125–150 m (r_{xy} = 0.028, P = 0.008, n = 177), 200–225 m (r_{xy} = 0.021, P = 0.047, n = 154) and 850–875 m (r_{xy} = 0.062, P = 0.044, n = 19; Figure 1). Correlation coefficients for the first distance classes did not differ from zero for females the second year (Table 2), however, there were weak positive correlations at 100–125 m (r_{xy} = 0.021, P = 0.006, n = 333), 125–150 m (r_{xy} = 0.021, P = 0.015, n = 269) and 525–550 m (r_{xy} = 0.029, P = 0.012, n = 144; Figure 1). The spatial structure for males was not significant in any of the distance classes starting at zero either year (Table 3).

3.4.3 Observational Data

Adult males moved their centroid farther from one year to the next than did adult females (178 ± 31 m *vs*. 79 ± 9 m, $t_{24,75} = 4.13$, $P < 0.001$), indicating that they were less sedentary than females. Mothers shifted their centroid by 75 ± 5 m on average from one 4-month period to the next ($n = 81$). Although offspring of both sexes were near their mothers at young ages, sons were located much farther from their mothers than were daughters between the ages of 18 and 25 months (Table 4, Figure 2). Daughters also moved away from their mothers as they aged but appeared to delay this movement until 26 months of age (Table 4). In addition to the sex and age effects, distances between mother-offspring pairs were shorter the second year (Table 5). Identity of the offspring contributed 23% to the overall variance in distances. Sizes of ranges varied according to sex-age class (95% kernel areas: ANOVA, $F_{6,204} = 21.20$, $P < 0.001$; 50% kernel areas: ANOVA, $F_{6,204} = 12.19$, $P < 0.001$; 95% kernel widths: ANOVA, $F_{6,204} = 21.51$, $P < 0.001$; 50% kernel widths: ANOVA, $F_{6,204} = 9.31$, $P < 0.001$; Table 6). Adult females, sub-adult females and young-at-foot of both sexes had 95% ranges with median width of 509–570 m. Large adult males and small males had much wider 95% ranges, with medians of 842 and 927 m, respectively. Sub-adult males had 95% ranges that were intermediate in width (median = 706 m). Median distance between the 4 known adult mother-daughter pairs was 206 m in 2011–2012 (Figure 3), so that three of four pairs overlapped their 95% ranges and two of four pairs overlapped their 50% core areas. Pair-wise relatedness (r) between adult mother-daughter pairs ranged from 0.407 to 0.648. There were no known adult mother-daughter pairs in the first year.

Mean relatedness (r) among adult females was low both years (–0.013 ± 0.002 in 2010–2011 ($n = 6{,}642$ pairs) and –0.010 ± 0.002 in 2011–2012 ($n = 11{,}130$ pairs)) and only weakly positive for those pairs of females with centroids within 25 m (0.065 ± 0.028, $n = 38$ in 2010-2011 and 0.028 ± 0.022, $n = 66$ in 2011–2012, Figure 4), confirming results of the local spatial autocorrelation analyses. Median distance between centroids of pairs of highly related adult females (relatedness coefficient $r > 0.45$) was 268 m ($n = 25$) in 2010–2011 and 243 m ($n = 42$) in 2011–2012. The distribution of pair-wise distances of adult females was such that 33% occurred in the 0–200 m classes, 9% in the 200–250 m class and 58% in the >250 m classes. Mean relatedness (r) among adult males was –0.026 ± 0.005 in 2010–2011 ($n = 1{,}406$ pairs) and –0.020 ± 0.003 in 2011–2012 ($n = 2{,}450$ pairs).

In total, 20 sub-adults and young adults (18 males and 2 females) were known to disperse from the study area. More males than females dispersed (34% of 53 males *vs.* 6% of 34 females, Fisher Exact Test, $P = 0.003$) but there was no difference in the median distance moved (2,019 *vs.* 2,486 m, respectively, $U = 15.0$, $P = 0.84$, n = 17 *vs.* 2). The greatest recorded dispersal distance

was 4,009 m. For 13 males of known birthdate, dispersal occurred at 24–63 months of age (mean = 43.3 ± 2.8 months). The single known-aged female that dispersed was 31 months old.

3.5 DISCUSSION

We found weak but significant fine-scale genetic structure among adult female kangaroos in both years but none among adult males either year. Immature male kangaroos moved away from their mothers at a younger age than did immature females. Also, a higher proportion of males than females were observed to disperse.

Non-random spatial grouping of female kin can result from sex-biased dispersal and social segregation without active preference for kin associates (Coltman *et al.* 2003). A previous study of eastern grey kangaroos found that females were philopatric at the fine scale but that female associations were only weakly related to kinship (Best *et al.* 2014). Because sub-adult females were combined with adult females in that study, however, it is unclear to what extent the associations were influenced by immature daughters, which often associate closely with their mothers. We restricted our analyses to adult females and found weak spatial genetic structure, even though females were sedentary from one year to the next. Because we combined behavioural observations with genetic analyses, we have an improved understanding of how the genetic structure among adult females arose. Adult daughters settled about 200–250 m away from their mothers but since one third of adult female pairs had centroids that were less than 200 m apart, the high density and adult survival rates affected temporal and spatial overlap of close kin such that females were unlikely to have close relatives as nearest neighbours. The positive genetic structure among females was thus likely a passive result of density and dispersal rather than active association.

Mammalian population genetic structure may be influenced by the extent of polygynous mating in addition to dispersal patterns (Stortz 1999). A rapid decline in fine-scale genetic structure was detected for red deer in conjunction with a sharp decrease in polygyny at high densities (Nussey *et al.* 2005). Male mating skew may be considerable in eastern grey kangaroos, with dominant males siring approximately 50% of juveniles in any one year in a population of about 55 individuals in a semi-captive environment (Miller *et al.* 2010). This high level of polygyny should result in daughters within a cohort being closely related through their fathers. The very restricted extent of genetic structuring found among the sedentary adult females in our study, however, indicates that male mating skew is likely much lower in free-ranging populations at high density.

Dispersal distances in mammals tend to correlate positively with body size but to be shorter for herbivorous species, so despite their relatively large size, kangaroos are expected to disperse a

median distance of about 3 to 5 km (Sutherland *et al.* 2000). Range size is likely a better predictor of dispersal distance than body size, with the median distance dispersed predicted to be 4.0–5.9 km based on adult ranges of 570–842 m width (Bowman *et al.* 2002). Kangaroos in our study appeared to disperse about 2–2.5 km but because we only collected opportunistic sightings along roads and tracks we may have missed long-distance dispersal events.

Dispersal distances can be affected by environmental conditions that likely vary from year to year (Slatkin 1987). Environmental effects influenced dispersal distances in a solitary rodent *Tamias striatus* such that females dispersed farther in years of favourable resource conditions and thereby increased the extent of positive genetic structure (Dubuc-Messier *et al.* 2012). Critically, the maximum extent of spatial genetic structure for chipmunk females occurred at a scale (50–250 m) that approximated or exceeded the diameter of individual home ranges (40 m), so that neighbours were likely to be close relatives (Dubuc-Messier *et al.* 2012). In contrast, female eastern grey kangaroos live in fission-fusion societies with overlapping ranges that are far wider (around 550 m) than the maximum extent of spatial genetic structure (50 m). As a result, female kangaroos in our population must mostly encounter non-kin. Any preferential associations found after accounting for range overlap will thus likely depend on factors other than kinship, such as reproductive state (Jarman & Southwell 1986) or sociability (Réale *et al.* 2007).

3.6 ACKNOWLEDGEMENTS

We thank G. Coulson, U. Gélin, E. Hynes and E. Rioux-Paquette for assistance with captures, and J. Chambers and E. Rioux-Paquette for genotyping. G. Coulson, A. Goldizen and F. Pelletier commented on previous versions of the manuscript. Funding was received from the Holsworth Wildlife Research Endowment, the Senate Research Committee of Bishop's University and the Natural Sciences and Engineering Research Council of Canada.

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Table 1. Results of global autocorrelations between genetic and geographic distances for adult female and male eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2010–2011, 2011–2012 and both years combined; r_{xy} = correlation coefficient according to Mantel tests; P = probability with 9,999 permutations; n = sample size. Significant correlations are in bold.

Sex	Year	r_{xy}	P	n
Female	2010–2011	**0.077**	**0.036**	82
	2011–2012	**0.095**	**0.008**	106
	Both	**0.079**	**0.012**	112
Male	2010–2011	0.028	0.31	39
	2011–2012	0.035	0.25	50
	Both	0.024	0.30	64
All adults	2010–2011	0.030	0.19	121
	2011–2012	**0.059**	**0.030**	156
	Both	0.030	0.14	176

Table 2. Results of autocorrelations between genetic and geographic distances for adult female eastern grey kangaroos in increasing distance classes at Wilsons Promontory National Park, Australia, 2010–2011 and 2011–2012; r_{xy} = correlation coefficient; P = probability with 9,999 permutations; n = number of pairs in each distance class. The x-intercept was set to zero if the correlation was not significant ($P > 0.05$). Significant correlations are in bold.

	2010–2012				2011–2012			
Distance class	r_{xy}	P	n	Intercept (m)	r_{xy}	P	n	Intercept (m)
0–15 m	**0.077**	**0.023**	18	51.0	0.017	0.30	23	0
0–20 m	**0.073**	**0.010**	29	47.1	0.015	0.26	46	0
0–25 m	**0.056**	**0.019**	38	53.2	0.013	0.27	66	0
0–50 m	0.016	0.12	137	0	–0.001	0.51	217	0
0–75 m	0.004	0.30	289	0	0.005	0.25	415	0
0–100 m	0.003	0.29	449	0	0.006	0.14	738	0

Table 3. Results of autocorrelations between genetic and geographic distances for adult male eastern grey kangaroos in increasing distance classes at Wilsons Promontory National Park, Australia, 2010-2011 and 2011-2012; r_{xy} = correlation coefficient; P = probability with 9,999 permutations; n = number of pairs in each distance class. The x-intercept was set to zero if the correlation was not significant ($P > 0.05$).

	2010–2012				2011–2012			
Distance class	r_{xy}	P	n	Intercept (m)	r_{xy}	P	n	Intercept (m)
0–15 m	–0.040	0.67	4	0	0.082	0.18	3	0
0–20 m	–0.046	0.73	5	0	0.082	0.18	3	0
0–25 m	–0.039	0.71	6	0	0.051	0.26	4	0
0–50 m	–0.007	0.58	23	0	0.010	0.40	13	0
0–75 m	0.000	0.50	38	0	–0.002	0.52	33	0
0–100 m	0.009	0.32	54	0	0.009	0.31	66	0

Table 4. Median distance (m) between the centroids of 85 eastern grey kangaroo mothers and their offspring of different ages at Wilsons Promontory National Park, Australia, April 2010–June 2012.

Age	Sons (n)	Daughters (n)	Mann-Whitney *U*	*P*
10–13 months	17 (47)	17 (34)	737.5	0.56
14–17 months	19 (53)	20 (36)	822.5	0.27
18–21 months	77 (45)	23 (23)	329.0	0.015
22–25 months	108 (38)	27 (19)	99.5	0.001
26–29 months	93 (24)	62 (16)	152.5	0.28
30–33 months	117 (17)	75 (13)	76.5	0.16
34–37 months	138 (15)	81 (11)	48.0	0.08
38–41 months	102 (6)	81 (7)	20.0	0.94

Table 5. Final model of a general linear mixed model of distances between centroids (log-transformed) of 85 mothers and 134 offspring for eastern grey kangaroos at Wilsons Promontory National Park, Australia, April 2010–June 2012, n = 404.

Coefficient	Estimate	Standard error	t	P
Intercept	1.294	0.058	22.35	<0.001
Sex (female)	–0.038	0.088	–0.43	0.66
Year (2011–2012)	–0.142	0.042	–3.42	0.001
Age (14–17 months)	0.182	0.072	2.52	0.012
Age (18–21 months)	0.555	0.077	7.18	<0.001
Age (22–25 months)	0.802	0.082	9.80	<0.001
Age (26–29 months)	0.836	0.098	8.51	<0.001
Age (30–33 months)	0.845	0.111	7.59	<0.001
Age (34-37 months)	0.991	0.116	8.56	<0.001
Age (38–41 months)	0.866	0.165	5.24	<0.001
Sex (female) X Age (14–17 months)	–0.117	0.112	–1.04	0.30
Sex (female) X Age (18–21 months)	–0.294	0.126	–2.34	0.020
Sex (female) X Age (22–25 months)	–0.370	0.134	–2.76	0.006
Sex (female) X Age (26–29 months)	–0.157	0.148	–1.07	0.29
Sex (female) X Age (30–33 months)	–0.169	0.164	–1.03	0.30
Sex (female) X Age (34–37 months)	–0.230	0.173	–1.33	0.18
Sex (female) X Age (38–41 months)	–0.035	0.227	–0.16	0.88

Table 6. Mean range sizes and widths based on 95% and 50% kernels for eastern grey kangaroos of different sex-age classes at Wilsons Promontory National Park, Australia, October 2010–June 2011. Sex-age classes with the same superscript did not differ in range size according to ANOVA and post-hoc Bonferroni multiple comparison tests on log-transformed data ($P > 0.05$).

		95% kernel				50% kernel			
Sex-age class	*n*	Size (ha)	SE	Width (m)	SE	Size (ha)	SE	Width (m)	SE
Small male	9	30.9^{a}	4.2	933^{a}	62	10.1^{a}	1.7	646^{a}	85
Large adult male	30	27.3^{a}	1.9	859^{ab}	35	8.7^{a}	0.5	515^{ab}	23
Sub-adult male	25	20.6^{ab}	1.5	714^{bc}	28	7.1^{ab}	0.7	426^{bc}	27
Sub-adult female	18	15.4^{bc}	1.4	624^{cd}	37	5.0^{bc}	0.6	367^{c}	31
Adult female	82	13.6^{c}	0.6	585^{d}	15	4.8^{c}	0.3	375^{c}	14
Young-at-foot female	19	12.3^{c}	1.1	560^{d}	25	4.2^{c}	0.5	350^{c}	28
Young-at-foot male	28	11.3^{c}	0.8	526^{d}	20	4.1^{c}	0.3	333^{c}	17

Supplementary Table S1. Microsatellite loci details for multiplex PCR amplification.

Locus	Genbank accession number	Annealing temperature (ºC)	No. of cycles	Dye	PCR recipe	Amount in multiplex (μl)	Multiplex No.
G12-6	AF322622	54	25	VIC	D	2.0	2
G16-1	AF322624	56	25	PET	D	2.0	2
G16-2	AF322625	54	30	PET	A	2.0	1
G19-1	AF322626	56	30	NED	B	2.0	1
G26-4	AF322628	56	30	VIC	B	1.0	2
G31-1	AF322629	56	25	NED	B	1.5	2
G31-3	AF322630	56	30	FAM	A	1.5	2
T3-1T	AF322644	56	27	FAM	A	1.0	1
T32-1	AF322654	54	30	VIC	C	1.0	1

Supplementary Table S2. Four polymerase chain reaction recipes for the 9 microsatellite loci used in Table S1. Concentrations are listed for a total reaction volume of 10μl.

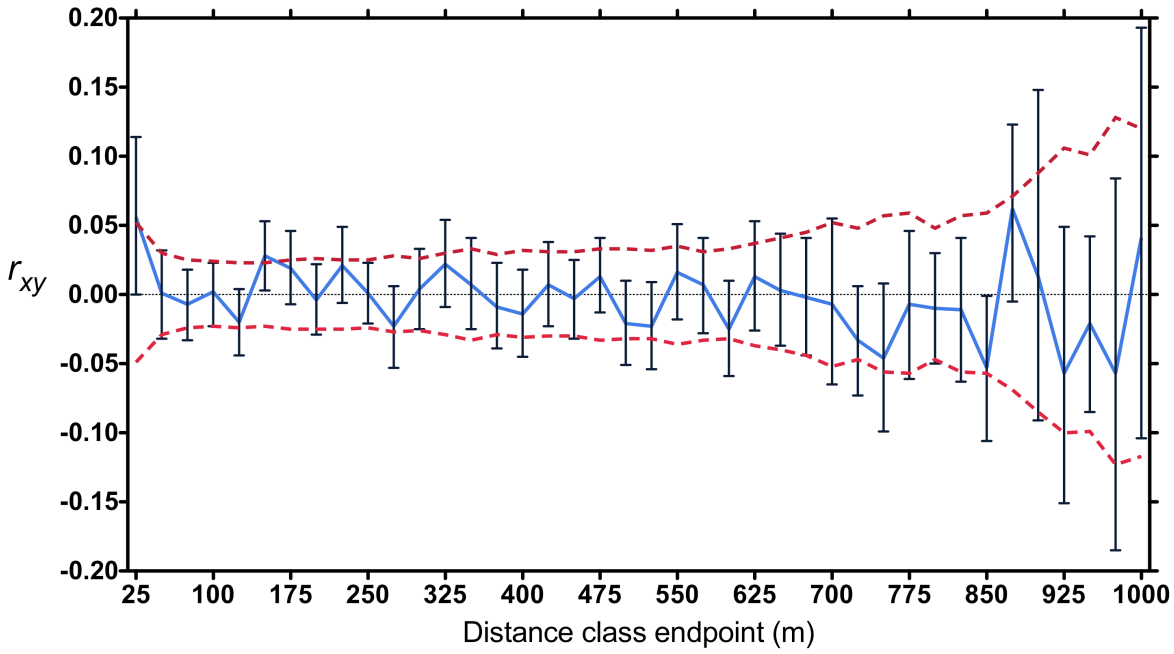
Product	A	B	C	D
dNTPs (mM)	0.2	0.2	0.2	0.2
$MgCl_2$ (mM)	2.5	2.5	2.5	2.5
10X reaction buffer (500mM KCl, 100mM tris-HCL)	1X	1X	1X	1X
BSA (mg/ml)	0.4	0.4	0.4	0.4
Fluorescently labeled forward primer (μM)	0.3	0.1	0.5	0.5
Unlabeled reverse primer (μM)	0.3	0.1	0.5	0.5
AmpliTaq Gold DNA polymerase (U) (Applied Biosystems)	0.35	0.5	0.25	0.5
DNA (ng)	10	10	10	10

Supplementary Table S3. Size range, number of alleles, observed (H_o) and expected (H_e) heterozygosity and probability of deviation from Hardy-Weinberg equilibrium (*P* H-W) for 9 microsatellite loci in 176 adult eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2010–2012.

Locus	Size range (bp)	No. of alleles	H_o	H_e	*P* H-W
G12-6	70–120	6	0.784	0.780	0.87
G16-1	140–190	6	0.812	0.708	0.13
G16-2	110–140	8	0.841	0.794	0.89
G19-1	165–225	9	0.795	0.789	0.56
G26-4	200–400	14	0.841	0.871	0.24
G31-1	105–145	10	0.824	0.798	0.66
G31-3	75–180	9	0.812	0.807	0.60
T3-1T	100–400	13	0.847	0.829	0.44
T32-1	140–195	9	0.744	0.713	0.78
Mean		9.3	0.811	0.788	

Figure 1. Correlograms for adult female eastern grey kangaroos at Wilsons Promontory National Park, Australia, in a) 2010–2011 and b) 2011–2012, comparing genetic distance to spatial distance in m, using 25-m distance classes. 'r_{xy}' refers to the correlation coefficient and is graphed in blue with 95% error bars from 10,000 bootstraps, while the 95% confidence intervals around $r_{xy} = 0$ are red dashed lines.

a



b

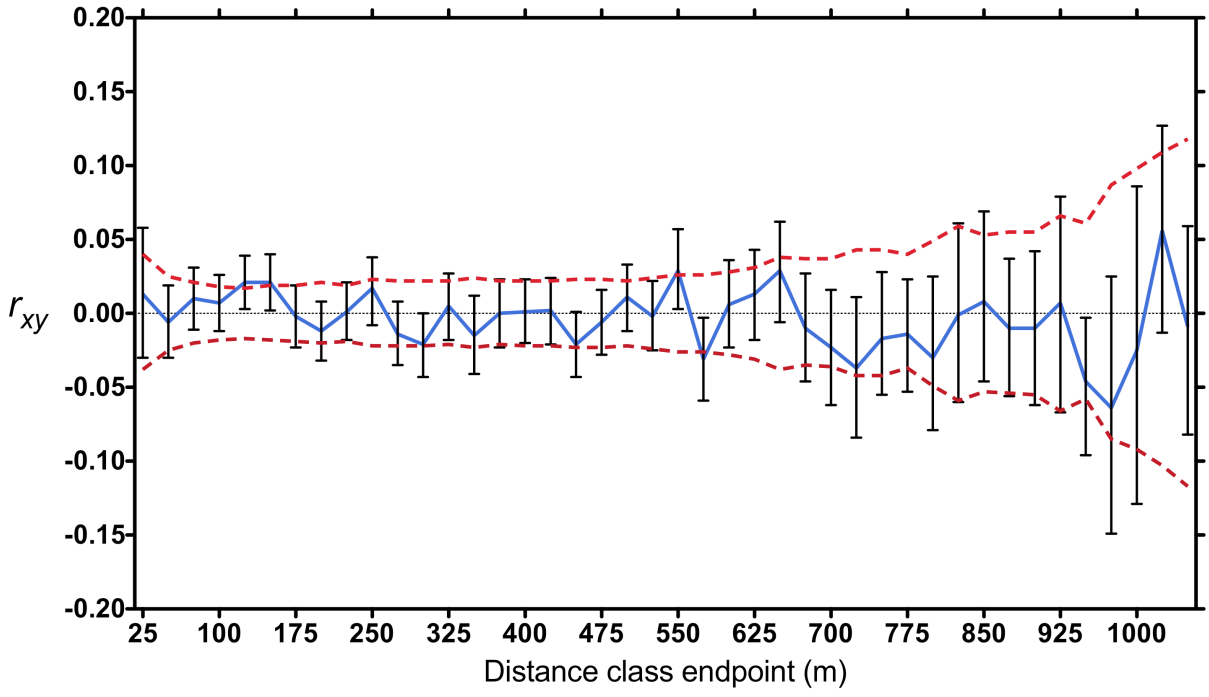


Figure 2. Distance between centroids of eastern grey kangaroo mothers and their offspring for 4-month age periods starting at 10 months of age, at Wilsons Promontory National Park, Australia, April 2010–June 2012. The solid line is a quadratic regression for sons (compared to a linear fit, $F_{1,242} = 19.46$, $P < 0.001$); the dotted line is a linear regression for daughters.

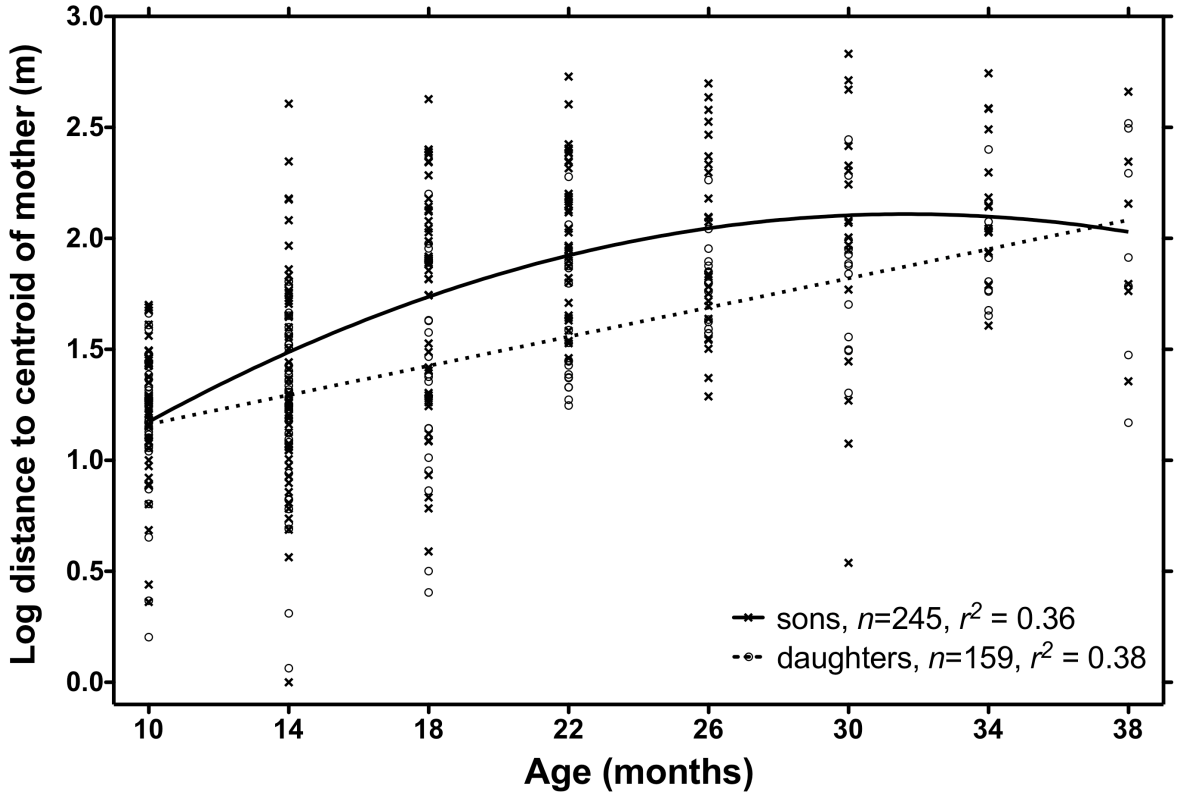


Figure 3. Map of 50 adult male (closed circle) and 106 adult female (open circle) centroids of marked eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2011–2012. The red lines connect centroids of 4 known mother-daughter pairs, the dashed line indicates the main road and the solid lines outline the arms of the grassy airstrip. The axes are the GPS coordinates with Y representing north.

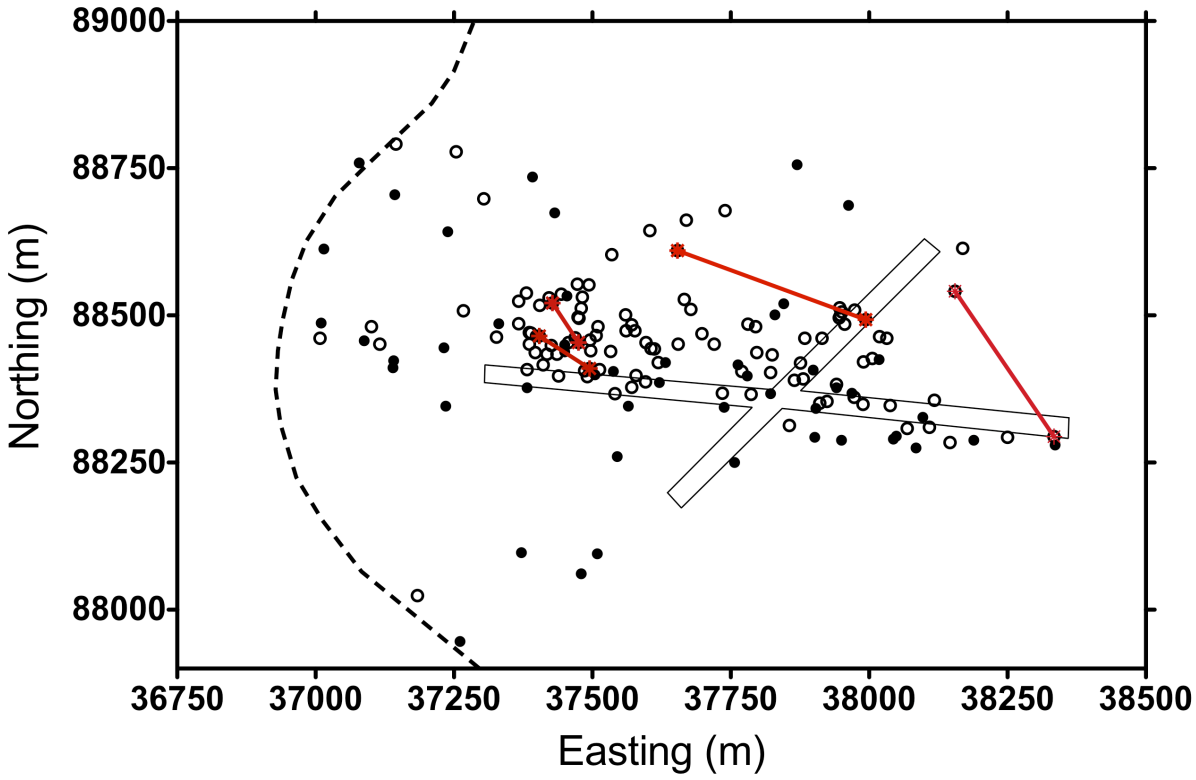
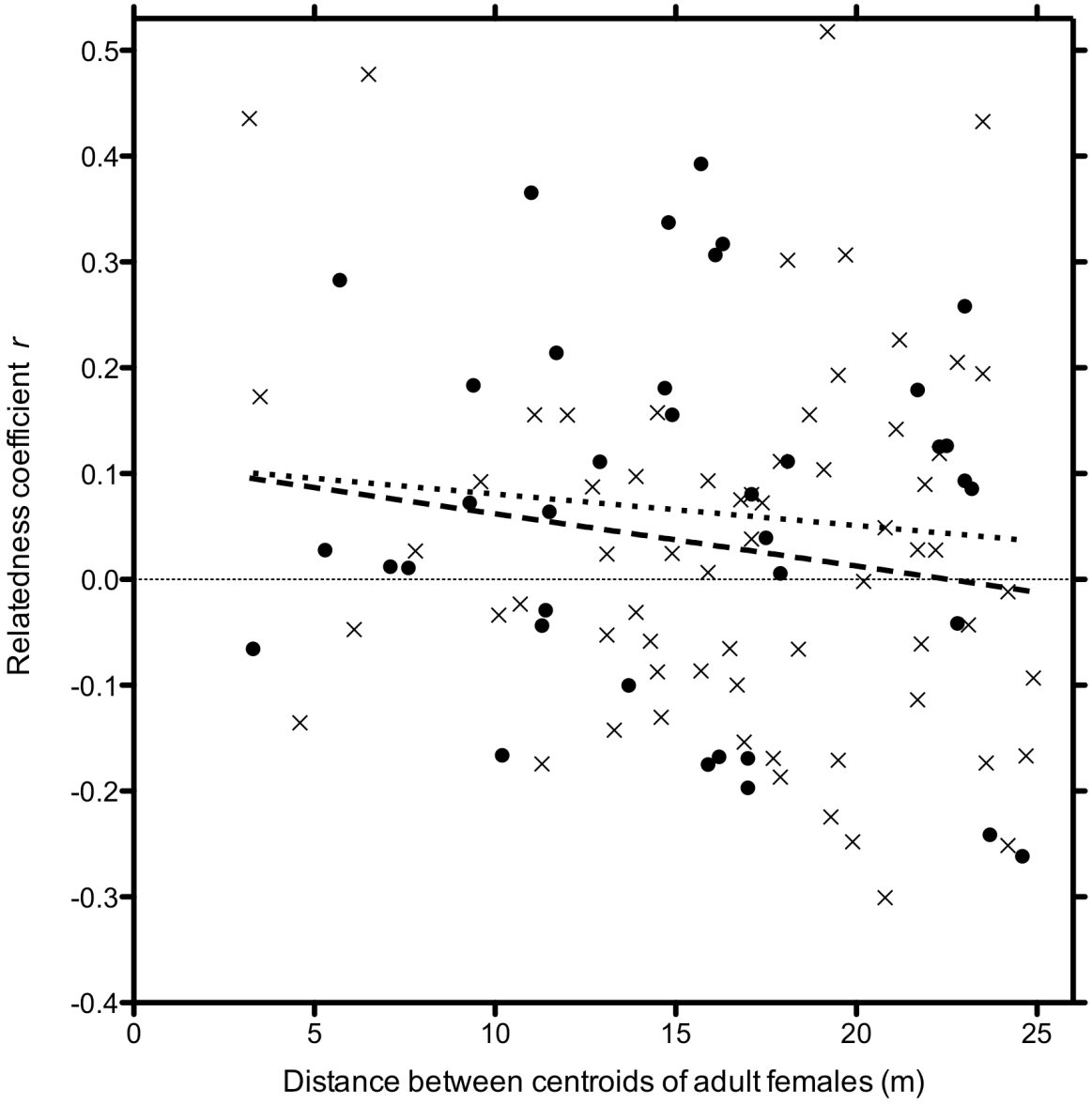


Figure 4. Pairwise relatedness coefficients *r* plotted against distance between centroids for adult female eastern grey kangaroos that had centroids within 25 m at Wilsons Promontory National Park, Australia, 2010–2011 (closed circles, $n = 38$) and 2011–2012 (crosses, $n = 66$). The dotted and dashed lines represent Pearson correlations for the two years ($r_p = 0.10$, $P = 0.56$ and $r_p = 0.15$, $P = 0.23$, respectively).



CHAPTER 4: Sex effects in the ontogeny of mother-offspring relationships in eastern grey kangaroos

Wendy J. King & Anne W. Goldizen

Manuscript to be submitted to *Animal Behaviour*.

4.1 ABSTRACT

Social relationships established early in life can have extensive effects on social structure and influence individual fitness. Eastern grey kangaroos *Macropus giganteus* nurse their young for at least 18 months, potentially allowing for a strong bond to develop between mothers and young. Because most female kangaroos are philopatric, the mother-offspring relationship established during lactation could persist into adulthood, resulting in clusters of female kin. Strong social bonds, however, require the establishment of affiliative behaviours and frequent interactions. In particular, one might not expect strong bonds among related individuals unless there are advantages to interacting with relatives compared to associating with unrelated conspecifics. We examined development of the mother-offspring relationship from permanent emergence from the pouch to the time of weaning in a high-density population at Wilsons Promontory National Park, Victoria. There were few differences in the behaviour of sons and daughters towards mothers (spatial measures, allogrooming, play, nosing and aggressive interactions), although daughters foraged slightly closer than sons to their mothers, and sons were weaned approximately two months earlier than daughters if the mother did not have a surviving large pouch young. Mothers associated more closely with their daughters (mean half-weight index (HWI) = 0.56) than their sons (mean HWI = 0.39) when offspring were aged 10–29 months but neither sex associated closely with their mothers beyond 33 months of age (mean HWI = 0.04). Mothers never intervened to defend their young from aggressive individuals and it was the offspring that maintained spatial proximity to their mothers. Kangaroo mothers had few interactions with their juvenile offspring other than nursing. Females may be philopatric and settle near close kin as adults but kangaroos appear to have few of those early affiliative interactions that are necessary for social bonds to develop.

4.2 INTRODUCTION

In species in which individuals are philopatric, kin of one or both sexes are likely to settle near each other and associate as adults (Clutton-Brock & Lukas 2012; Michener 1983). Kin may

thus form clusters within a population and behave cooperatively (Hamilton 1964), which can lead to kin selection (Maynard-Smith 1964). Social relationships established early in life among kin can have extensive effects on social structure and influence individual fitness (Viblanc, Arnaud, Dobson & Murie 2010). Many species of mammals have social systems based on female kinship, *e.g.* African elephants *Loxodonta africana* (Archie, Moss & Alberts 2006), spotted hyenas *Crocuta crocuta* (Holekamp, Smith, Strelioff, Van Horn & Watts 2012), yellow baboons *Papio cynocephalus* (Silk, Altmann & Alberts 2006) and bottlenose dolphins *Tursiops aduncus* (Frère et al. 2010). Frequent interactions, such as allogrooming, appear necessary to maintain bonds among kin in many species of primates (Silk 2002). These bonds often occur between mother and offspring and close associations may develop when offspring are young and still dependent on their mothers (Silk 2002). If females tend to be philopatric and have preferential associations with kin as adults, daughters may be expected to develop stronger bonds than sons with their mothers (Greenwood 1980).

There are various accounts of the development of mother-offspring relationships in eutherian mammals *e.g.* rhesus monkeys *Macaca mulatta* (Suomi 2005), vervet monkeys *Chlorocebus aethiops* (Hauser & Fairbanks 1988), South African giraffes *Giraffa camelopardalis giraffa* (Langman 1977), bottlenose dolphins (Mann & Smuts 1999; Mann & Watson-Capps 2005) and humpback whales *Megaptera novaeangliae* (Szabo & Duffus 2008). Sex-specific social patterns developed early in life predict long-term bonds in vervet monkeys (Fairbanks & McGuire 1985), baboons (Pereira 1988) and dolphins (Stanton, Gibson & Mann 2011). Vervet monkey mothers approach and groom their juvenile daughters more than their sons, and females subsequently form cooperative female kin groups as adults (Fairbanks & McGuire 1985). Daughters preferentially approach and spend time with adult females, including their mothers, in both vervet monkeys and baboons, which is consistent with the hypothesis that juveniles prefer the proximity of potential future alliance partners (Fairbanks 1993; Pereira 1988). Male dolphin calves form stronger ties than expected with same-sex calves and males subsequently form long-term alliances as adults (Stanton et al. 2011).

Few studies of mother-offspring relationships have focused on marsupial mammals. In marsupials, gestation is short and lactation is prolonged compared to eutherians (Tyndale-Biscoe & Renfree 1987). One of the largest marsupial species, the eastern grey kangaroo *Macropus giganteus*, nurses its young for at least 18 months after birth, with weaning occurring about 8 months after the offspring has permanently left the pouch (Poole 1975). Adult kangaroos are highly dimorphic (Jarman 1989) and there is likely greater variation in reproductive success among males than females (Jarman 1983). The early mother-offspring relationship is characterised by the offspring closely following the mother and interactions comprise greeting, allogrooming and play,

with little evidence of agonistic behaviour of mothers towards young at the time of weaning (Russell 1989). Because female kangaroos appear to be philopatric (Chapter 3), the mother-offspring bond that occurs during lactation may persist into adulthood, resulting in clusters of female kin that form temporary feeding groups. Indeed, there is evidence that some adult females associate with close kin, although this pattern is not strong (Best, Dwyer, Seddon & Goldizen 2014; Jarman 1994). Availability of close kin could affect a female's ability to access resources, avoid predation and care for her offspring if female kangaroos exhibit kin-preferential behaviour (Clutton-Brock 2002), however female kangaroos do not appear to exhibit cooperative behaviour as adults (Best, Seddon, Dwyer & Goldizen 2013). Because eastern grey kangaroos are one of the most social marsupials with a social system composed of loose associations within a fission-fusion society (Jarman & Southwell 1986), they are an ideal species for investigating the importance of mother-offspring relationships in mammalian social structure (Aureli et al. 2008).

Here we examine development of the mother-offspring relationship from permanent emergence from the pouch to the time of weaning in a high-density population of eastern grey kangaroos. We predicted that females would have closer and more affiliative relationships with their daughters than with their sons because, as suggested by Best et al. (2014), adult females may associate with relatives, and if so, females could become familiar with relatives at a young age and then extend these relationships into adulthood (Viblanc et al. 2010). We specifically examined how mother-offspring relationships developed over time and whether females had closer and more amicable relationships with their daughters than their sons as quantified by spatial measures, amounts of different behavioural interactions, frequency of distress calls and time spent together. We also compared vigilance rates of females with daughters compared to sons.

4.3 METHODS

4.3.1 Study Site, Population and Captures

The 110-ha study site at Wilsons Promontory National Park (38° 57' S, 146° 17' E) was centred on a grassy landing strip and surrounding meadows. There were no wild dogs but predators on juveniles included red foxes *Vulpes vulpes* and wedge-tailed eagles *Aquila audax*. Between about 500 and 800 kangaroos inhabited the study area, depending on the year, and there were 80 to 125 marked adult females each year. This population had been studied since 2008, so the kangaroos were habituated to human presence. Adult females were marked for visual identification with plastic collars and plastic eartags (King, Wilson, Allen, Festa-Bianchet & Coulson 2011). Offspring were marked with small plastic eartags, usually while still in the pouch. Most captures to mark pouch young took place in late winter/early spring. Birthdates were derived from body

measurements (mean date based on length of the head, hind leg and hind foot) taken when the young were marked in the pouch and calculated for males and females separately (Poole, Carpenter & Wood 1982). Most offspring were marked at the age of 8 or 9 months. Body conditions of mothers and young were estimated when the mothers were captured and the pouch young were marked, as the standardised residual of the linear relationships between hind leg length and log body mass (Schulte-Hostedde, Zinner, Millar & Hickling 2005). Females do not reach reproductive maturity until about 36 months of age in this population (Chapter 3).

Captures were undertaken with ethics approval from the University of Melbourne (#0810628.1 and #0911512.1) and research permits from the Victorian Department of Sustainability and Environment (#1004582 and #1005558). Observations were conducted with animal ethics approval from the University of Queensland (#SIB/206/09/(NF)).

4.3.2 Focal Sampling

We made focal animal observations (Altmann 1974) on 25 mother-offspring pairs (13 daughters and 12 sons) between September 2010 and December 2011 and on 30 mother-offspring pairs (13 daughters and 17 sons) between September 2011 and June 2012. Observations on the first cohort (born 2009/2010) were extended because some individuals were observed to be suckling beyond 18 months. Observations on the second cohort (born 2010/2011) ceased on a set date, when 80% of offspring were at least 17 months old. There were 174 focal observations on sons ($X = 6.0 \pm 0.4$) and 191 on daughters ($X = 7.3 \pm 0.5$). Each pair was observed 3 to 12 times and 5 individual mothers were watched in both years. Offspring ages were estimated to 0.1 month, using birthdates calculated from sex-specific curves in Poole et al. (1982). Observations usually began when the offspring was 10 or 11 months of age and had permanently emerged from the pouch, and were repeated monthly until offspring were no longer found with their mothers or until 21 months of age, whichever came first, except for the second cohort. The mean age of young across all focal observations was 14.6 ± 0.1 months.

Focal samples took place in the early morning or late afternoon, when most animals were actively foraging. Each pair was followed for 5 min from a median distance of 24 m (range 7 to 121 m) using 8X32 binoculars (Leitz, Germany) and always began when the mother was foraging. Observations were split evenly between the two times of day (190 in the morning and 175 in the afternoon). Wind speed was measured over 5 min using a Kestrel 2000 anemometer (Nielsen-Kellerman, Boothwyn, PA) and the mean and maximum speed recorded ($X = 5.2 \pm 0.2$ and 11.0 ± 0.5 km/h, respectively). The observer (WJK) noted all behaviours of both individuals continuously and the distance between the pair (in m) at the start and at 1-min intervals. Behaviours were

categorised as feeding (biting and chewing in crouched position with head below horizontal, including pentapedal movement while feeding), vigilant (head raised above horizontal in crouched or upright position and scanning (according to the criteria of Pays and Jarman (2008)), grooming (scratching and/ or licking body), moving (bipedal hopping or pentapedal stepping), resting (lying, sitting crouched or standing upright with no head movement) and interacting. A bout consisted of a continuous behaviour and ended when the behaviour changed. When one member of the focal pair hopped off and left a group, the observer noted whether the other member followed within a few seconds and the observation continued if both members of the pair remained within sight. Most observations (84%) were of mother-offspring pairs that remained together in the same group for the entire focal sample. Sometimes the offspring attempted to suckle but the mother moved away while continuing to feed, which was tallied as a suckle attempt. If the mother stopped feeding to allow the offspring to suckle, however, we abandoned the focal observation and omitted it from analyses. Suckles generally last 11–15 min (Stuart-Dick 1987) and including them would have biased the calculation of maternal time budgets.

We also recorded each time one member of the pair approached or retreated from the other, with movements separated by pauses of at least 4 sec considered as separate movements. The number of retreats and approaches ranged between 0 and 11 for mothers and 0 and 10 for offspring per 5-min period. From these data, we calculated Hinde's proximity index (Hinde & Atkinson 1970):

$$\frac{Ap_o X\ 100}{Ap_m + Ap_o} - \frac{R_o X\ 100}{R_m + R_o}$$

where Ap = approaches, R = retreats, o = offspring and m = mother. The index ranges from –100 when the mother is fully responsible for maintaining proximity, to +100 when the offspring is fully responsible. The left-hand side of the equation is the percentage of all approaches that were undertaken by the offspring while the right-hand side is the percentage of all retreats undertaken by the offspring. When there were no approaches (or retreats) by either member of the pair, the approaches and retreats did not add up to 100%.

Additional variables recorded that could potentially affect kangaroo behaviour while foraging included group size, maximum wind speed and time of day (morning or evening). A group consisted of a set of animals in which each individual was within 10 m of at least one other (10-m chain rule (Jarman 1987)), and within visual contact of at least one group member. When group composition changed, we calculated group size as a mean based on the proportions of the 5-min period that the focal pair spent in different-sized groups. When the pair was not observed in the same group, we used the group size of the mother. Each offspring was given a unique identity number and because about 50 offspring were marked each year, the observer usually did not recall

the sex of the young. Observational bias due to knowledge of the sex of the young was therefore minimised (Tuyttens et al. 2014).

4.3.3 Instantaneous Sampling

Instantaneous sampling (Altmann 1974) involved one observer (WJK) walking slowly around sections of the study area for about 45 h/month, just after dawn and before dusk (during periods when animals were actively foraging) for 10 to 12 consecutive days each month between April 2010 and June 2012 (total 1,122 h). A second observer attempted captures in another section of the study area at the same times from July 2010 to August 2011, in October 2011 and in March 2012 and took additional observations only if the animals were undisturbed. This observer provided the data on approximately 25% of the total 17,748 groups recorded. We recorded group size (including unmarked individuals) and group composition of marked individuals. From these observations, we estimated the proportion of time mothers and offspring spent together as half-weight indices (HWIs) calculated over 4-month time periods in SOCPROG 2.4 (Whitehead 2009), if both individuals were seen at least 10 times/period. These periods were based on the ages of the offspring, starting at 10 months, and so were calculated over a moving time window for 138 mother-offspring pairs.

4.3.4 All Occurrences Sampling

Because interactions were rare and of short duration, we used 'all occurrences sampling' (Altmann 1974) to quantify interactions, rather than only using those tallied in focal observations. We thus recorded interactions while conducting instantaneous sampling surveys of the population. We recorded interactions between 103 marked adult females and their 148 juvenile offspring up to 21 months of age and also interactions between 55 marked (and about 25 unmarked) adult females and 79 marked non-offspring juveniles up to 21 months of age. Interactions included 1) nursing, 2) allogrooming, 3) playing with the other's head, shoulders and back, 4) nosing the other's body or head (greeting) without further interaction and 5) aggressive interactions where the adult female growled at, batted and/or hopped at young (Coulson 1997). We also noted all occurrences of distress or 'isolation' calls (Coulson 1997) given by young up to 21 months of age, together with the young's group size, whether the mother was present in the same group or separated from the young, and the distance between the pair. Allogrooming, play and distress calls were only counted once per mother-offspring pair per observation session but nursing and aggressive interactions were occasionally counted a second time within a session if they were repeated after at least 10 min.

Nosing without further interaction was a subtle interaction and was not seen to be repeated within a session.

We calculated individual rates for the different interactions and distress calls over three 4-month time periods when offspring were 10–13 months, 14–17 months and 18–21 months, by dividing the number of occurrences by the number of days each offspring was seen. These rates included the few play, allogrooming and aggressive interactions recorded in focal observations (6, 4 and 3 interactions, respectively). We excluded the nosing interactions seen in focal observations because they were much more subtle interactions and unlikely to be seen in all occurrence observations. We observed no distress calls during focal observations. For the same 3 periods, we also noted whether or not an offspring was seen suckling each day it was observed, including both focal observations and all occurrences. We also noted the date that each offspring was last seen in its mother's pouch.

4.3.5 Statistical Analyses

We carried out linear mixed-effects models using offspring identity as a random factor with R version 2.15.2 (R Development Core Team 2012). Response variables were mother-offspring HWI, proportion time mothers spent vigilant, mean distance between the pair, Hinde's proximity index and the likelihood of offspring being seen suckling. Predictors were offspring sex, age and cohort and other factors believed to influence kangaroos' behaviour during feeding bouts, including body conditions of both individuals, time of day, maximum wind gust speed and group size. Body condition indices were unavailable for a few offspring that were first caught as young-at-foot (rather than in the pouch), so initial analyses were run on a subset of observations for which body conditions were known; these are termed the complete models in results. Subsequent analyses omitting body condition were run on a larger dataset. Proportion time mothers spent vigilant and mean distance between the pair were checked for normality and transformed (arcsine square root and logarithm, respectively) to improve distribution before examination using a linear mixed-effect model fit with restricted maximum likelihood (REML). Results of the models are presented back-transformed. Because of our inability to transform some variables to normal distributions, the likelihood of Hinde's proximity index being positive or negative or of the offspring being seen suckling or not were analysed using generalised linear mixed-effect logistic regression and binomial errors. Predictors for the analysis of whether offspring were seen suckling only included offspring age and sex, cohort and body conditions of the offspring and mother. The response variable of HWI was analysed using offspring sex, age and cohort as predictors. We checked for colinearity between predictors using variance inflation factors (VIFs) from linear models (Quinn & Keough

2002). All VIFs were less than 4. We sequentially removed the least significant parameter (based on its *P*-value, threshold ≥0.05) from the model using stepwise backward selection (Crawley 2007). Initial models included interactions between offspring age and sex and between offspring age and time of day. Finally, we examined quartile-quartile plots of the residuals of chosen models to check for departure from normality (Crawley 2007). To visually represent how HWIs declined with age of the offspring, we performed segmental linear regression, which is a combination of two linear regressions for different parts of the x-axis (Faraway 2002).

Because of the lack of normally distributed datasets and small sample sizes, rates of interactions and distress calls were compared for sons and daughters using Mann-Whitney *U*-tests following Kruskal-Wallis tests for differences across 3 age periods. Likelihood of following the mother or offspring was analysed by sex and age of the leader using Fisher Exact Tests. We also used Fisher Exact Tests to compare the proportions of males and females seen suckling after 18 months of age. Sex differences in age last seen suckling and age last seen in the pouch were tested using unpaired *t*-tests. Age last seen suckling was calculated separately for offspring whose mothers did and did not have a subsequent offspring that survived. Means are presented ± 1 standard error of the mean.

4.4 RESULTS

4.4.1 Birth Dates and General Behaviour of Mothers

Births were seasonal, with 77% occurring in late spring and summer (November through February; Figure 1). Only 3/189 juveniles were estimated to have been born in the winter months of June, July and August and therefore young were divided into cohorts at 1 July.

On average, mothers spent 90.7 ± 0.5% of their time feeding and 6.0 ± 0.4% vigilant during the focal samples, which were undertaken during foraging periods. Other behaviours (grooming, moving, resting and interacting) only made up 3.3% of the time budget. Mothers fed for 3.5 ± 0.1 bouts during 5-min focal samples, which were interrupted by 2.3 ± 0.1 bouts of vigilance, on average. Sex and age of the offspring had no effect on proportion of time vigilant but vigilance decreased with group size, increased with maximum wind speed and was higher for mothers of the second cohort of young (Table 1). Foxes were sighted 5 times more frequently the second year than the first (0.75 *vs*. 4.24 per 100 h observations). Individual identity contributed 15% to the overall variance in maternal vigilance.

4.4.2 Sex Difference in Spatial Measures

Daughters were found to forage about 1 m closer to mothers than sons and the distance increased as the young aged (Table 2). Median mean distance between mothers and their offspring was 3.3 m and was much greater in the evening than the morning, with an interaction between time of day and offspring age so that the distance increased as the young aged from about 2 m to 5 m for the morning only (Table 2). Mean distance was also shorter on windy days and for the first cohort (Table 2). The identity of the young contributed <1% to the overall variance in distance between young and the mother.

Daughters were not more responsible for maintaining proximity to their mothers than sons because sex of the young did not affect Hinde's proximity index ($z = 0.55$, $N = 277$, $P = 0.58$ in the complete model including body condition). The proximity index decreased with offspring age and was more likely to be positive in the morning than the afternoon (Table 3). On average, the index was positive and so proximity was primarily maintained by the offspring (X proximity index = 20 ± 3).

There was no difference between the sexes for whether offspring followed their mothers (96% for daughters *vs*. 91% for sons) or whether mothers followed their offspring (20% for daughters *vs*. 0% for sons, Fisher Exact Tests: $N = 48$ or 26, $P > 0.22$), when one member of the focal pair suddenly left a group. Mothers followed offspring on only 12% of 26 occasions, while offspring followed mothers on 94% of 48 occasions (Fisher Exact Test: $N = 74$, $P = 0.001$). Group sizes during focal samples ranged from 1 to 14 ($X = 3.2$) and 35% of mothers' groups experienced changes in composition over the 5-min focal observation period. There were 193 changes in 365 groups, which represented a change every 9.4 min.

4.4.3 Sex Difference in Age Last Suckling and in the Pouch

Young were seen to suckle up to the age of 23.1 months, however only 15% of 47 sons were seen suckling after 18 months, compared to 43% of 28 daughters (Fisher Exact Test, $P = 0.012$). If the mother had a subsequent offspring that survived to large pouch young stage (LPY) over the next 12 months, daughters were last seen suckling at the same age ($X = 16.4 \pm 0.8$ months) as sons ($X = 16.1 \pm 0.3$ months, Unpaired t test: $t_{7,29} = 0.50$, $P = 0.62$). However, mothers nursed their daughters to an older age than sons if they did not have a surviving LPY (for daughters $X = 18.9 \pm 0.6$ months *vs.* for sons $X = 16.5 \pm 0.8$ months, Unpaired t test: $t_{19,17} = 2.52$, $P = 0.016$). Nursing rates declined as young aged, with a cohort effect, and confirmed that there was an interaction between age and sex of the young, presumably because sons were weaned earlier than daughters (Figure 2, Table 4). It was often difficult to determine which member of the pair approached the other to initiate nursing

but it was the offspring on 76% of 51 occasions when the initiator was clear. Group sizes during instantaneous sampling ranged from 2 to 35 and mothers were alone with their young for 50% of 410 nursing events.

There was no difference between daughters and sons for the age last seen in the pouch (X = 10.1 ± 0.1 months for both, Unpaired t test: $t_{38,50} = 0.81$, $P = 0.42$).

4.4.4 Sex Difference in Amounts of Affiliative and Aggressive Interactions with the Mother

Rates of allogrooming and play with the mother decreased with age of offspring, while aggressive interactions increased (Table 5). There were no differences, however, between sons and daughters in rates of allogrooming, play, nosing or aggression with the mother at any age (Table 6). Offspring initiated all play bouts for which we could determine the initiator ($N = 33$). Mothers and offspring had approximately similar numbers of aggressive and nosing interactions ($N = 29$ *vs.* 46 interactions).

Juvenile kangaroos were exposed to about 7 times more aggressive than non-aggressive interactions from females that were not their mothers ($N = 125$ *vs.* 17 interactions). Allogrooming and play did not occur between adult females and non-offspring. Mothers did not intervene when their offspring were attacked by other females. Juveniles were most likely to be attacked if their mother was not in the group (69% of 114 times attacked). Young always moved away from aggressive adult females and often hopped away (51% of 53 times attacked where the result was noted). Sometimes young moved towards their mothers (54% of 33 times attacked if their mother was alive and her location was known).

4.4.5 Sex Difference in Frequency of Distress Calls

Sons and daughters gave distress calls at similar rates (Table 6) and calling frequency decreased with offspring age (Table 5). In 95% of 116 cases when distress calls were observed, the young were separated from their mothers (X distance = 40 ± 4 m, $N = 66$) and often alone in a group size of 1 (63%). In 83% of 70 occasions when the young called and the mother was seen nearby, she did not react by approaching. The maximum known distance between a calling young-at-foot and its mother was 188 m. Of the 27 occasions when pairs were seen to come together following a distress call, it was the young that approached 70% of the time, with nursing occurring immediately following the reunion 37% of the time.

4.4.6 Sex Difference in Time Spent with the Mother

Time spent with the mother decreased as young aged and was higher for daughters than sons, with an interaction between sex and age (Table 7, Figure 3). Daughters only spent more time with their mothers at 14–17 and 18–21 months of age (Table 7). Neither sex associated closely with their mothers beyond 33 months of age (mean HWI = 0.04). Mean HWI at 18–21 months for those young known to be nursing beyond 19 months was high (0.68, $N = 11$) compared to others (0.27, $N = 57$), indicating that unweaned young likely spent more time with their mothers than weaned young of the same age. Individual identity of the young contributed 43% to the overall variance in HWI.

4.5 DISCUSSION

Contrary to our prediction, mothers did not show much closer relationships with their daughters than with their sons in terms of affiliative interactions, at least during foraging periods. However, mothers spent more time with young daughters than young sons, foraged slightly closer to daughters than sons and sometimes nursed daughters to an older age than sons. Mothers did not display a protective parenting style at the young-at-foot developmental stage; they spent little time vigilant, never intervened to defend young from aggressive individuals and rarely approached young emitting distress calls. Mothers spent about 90% of their time feeding during focal observations, suggesting that they had little time available to devote to vigilance and monitoring of their offspring. The proportion of time that mothers spent vigilant was consistently very low compared to other studies (6% *vs*. around 15%) (Colagross & Cockburn 1993) and could reflect weak predator pressure. However, vigilance was higher the year when there was an apparently higher fox presence and vigilance decreased with group size. The overall low vigilance and high feeding rates were more likely due to females competing for food at the high density rather than weak predator pressure. Females with pouch young or young-at-foot were found to have higher bite rates than other adult females in this population (Gélin, Wilson, Coulson & Festa- Bianchet 2013).

Young-at-foot maintained a distance of 2–5 m from their mothers and therefore usually remained within the same group, but occasionally joined other groups. Another study on kangaroos found mother-offspring distances averaged 4 m (Stuart-Dick 1987) at the young-at-foot stage. This developmental stage is equivalent to the entire suckling stage of eutherian young since permanent emergence from the pouch in marsupials has been likened to birth in eutherian mammals (Tyndale-Biscoe & Renfree 1987). A similar lack of developmental change in mother-offspring distances has been found for bison *Bison bison* calves (Green 1992). It is possible that young kangaroos abruptly cease associating closely with their mothers at weaning, perhaps because by this stage the mother

often has another offspring ready to leave the pouch (Poole 1975). Sons were not often found in the same group as their mothers beyond 21 months (Figure 3) and started to move away from their mothers at 18 months (Chapter 3), the average age of weaning. In contrast, daughters continued to associate closely with their mothers until about 26 months (Figure 3). It thus appears that most sub-adult daughters remained associated with their mothers beyond weaning. In several mammalian species that exhibit tight mother-offspring pairs with the offspring following their mothers, young separate spatially from mothers at weaning, *e.g*. humpback whales (Szabo & Duffus 2008).

It was clearly the kangaroo offspring that was responsible for the mother-offspring association during the young-at-foot stage by maintaining proximity with its mother and following her when she hopped away from a group. The lack of a sex effect in either behaviour suggests that selection pressures to remain with their mother are strong for both daughters and sons before weaning. Hinde's proximity index was found to be less appropriate than the likelihood of following for describing developmental patterns in mother-offspring relationships in bison (Green 1992) and other ungulates in general (Ralls, Lundrigan & Kranz 1987), likely because the number of approaches and retreats were low. We also found a stronger indication of which member of the mother-offspring pair maintained the association using the likelihood of following rather than Hinde's index.

Mothers nursed daughters to a later age than sons, but only if they lost their subsequent pouch young before the LPY stage. Daughters were also more likely to be seen suckling than sons at the oldest young-at-foot age but this likely represents extended nursing rather than an increased rate of suckling because sons were mostly weaned by this age. We do not know whether extended nursing of daughters would represent increased investment in daughters compared to sons, however we would have expected mothers to invest more in sons because of the higher potential reproductive success of sons (Trivers & Willard 1973). Kangaroo mothers did not initially invest more in one sex than the other, as measured by age of the young at permanent pouch emergence, although sons may have received more milk than daughters while in the pouch, since sons grow at a slightly faster rate (Poole et al. 1982) and mothers of sons had a higher bite rate than mothers of daughters in this population (Gélin et al. 2013). Red deer *Cervus elaphus* and African elephant mothers invest more in sons than daughters as measured by the frequency and duration of nursing bouts (Clutton-Brock, Albon & Guinness 1981; Lee & Moss 1986). Both of these species are highly dimorphic and mating success of sons depends on fighting ability, as appears to be the case in kangaroos (Jarman 1983). Perhaps kangaroo mothers that nurse daughters to an extended age following the loss of a subsequent pouch young are 'making the best of a bad job' and continuing to invest in a previous reproductive event until the next breeding season. Mothers may not continue to nurse sons to a late age because they are larger than daughters and too costly at this stage of

development. Suckle bout duration and frequency are not closely correlated with milk intake in many species, however, and whether suckling at older ages involves significant milk transfer is unknown, particularly due to the possibility of non-nutritive sucking (Cameron 1998). In addition, kangaroo sons increase movements away from their mother at a younger age than daughters (Chapter 3) and may wean themselves at a younger age. Our focal observations only took place on pairs that were seen at the same time; offspring that did not associate with mothers could not be observed simultaneously with their mothers. Mammalian sons are often more active than daughters and this sex difference affects the closeness of the mother-offspring relationship from 3 months of age in giraffes (Pratt & Anderson 1979) and for neonates in yellow baboons (Nguyen, Gesquiere, Alberts & Altmann 2012).

There was no evidence for sex differences in rates of allogrooming, play, nosing, aggressive interactions or distress calls. It thus seems that daughters and sons behave similarly towards their mothers when young-at-foot, at least during foraging periods. These results concur with those in yellow-bellied marmots *Marmota flaviventris* (Smith, Chung & Blumstein 2013) but contrast with findings in many primate species in which sex-differential juvenile behaviour occurs (Cords, Sheehan & Ekernas 2010; Pereira & Fairbanks 2002). Eastern grey kangaroos are highly dimorphic as adults (Jarman 1989), but sex-differential social behaviour may not occur before weaning or adulthood. Female kangaroos mature at age 3 in our population (Chapter 3) and males at age 4 (Poole & Catling 1974), meaning that there is a sub-adult stage of approximately 18 months in females and 30 months in males. Alternatively, because our analyses revealed no differences in the social behaviour of sons and daughters between the ages of 10 and 21 months, it is possible that males and females display similar social relationships as adults, with the exception of mating activities. Jarman & Southwell (1986) noted that kangaroo grouping patterns tend to be homogeneous across the sexes, unlike the societies of similar-sized ungulates.

We found little evidence of bonding behaviours, such as allogrooming, between mothers and young. In another study on eastern grey kangaroos, mothers spent more time grooming their small young-at-foot daughters than their sons (70% *vs*. 45% of contact time), but similar proportions of time nursing sons and daughters (Stuart-Dick & Higginbottom 1989). We conducted observations while most animals were feeding in the early morning and late afternoon and it is possible that mothers treat daughters differently from sons in resting periods, although females seldom interact while resting and tend to groom offspring during nursing bouts (Coulson 1997). Focal observations of mother-offspring pairs were abandoned if nursing occurred and we did not time allogrooming events, however all occurrences of nursing and allogrooming were tallied during our population surveys, so it was unlikely that we overlooked occasions of maternal grooming of young. It is also possible that mothers treat daughters and sons differently prior to permanent emergence from the

pouch. Due to the sampling schedule of 10-12 days per month, it was impossible to consistently observe mother-offspring pairs in the 5-week period when offspring are frequently in and out of the pouch (Poole 1975). However, the dynamic in-and-out stage lasts only about 1 month compared to the subsequent 8 months of lactation by young-at-foot.

Another mammalian species that displays a similar social structure with fission-fusion grouping, associations among female kin (Frère et al. 2010), and following behaviour of juveniles (Mann & Smuts 1999) is the bottlenose dolphin. Although proximity between mother and newborn dolphins gradually decreases over the first 10 weeks, there is much social contact between such pairs, often involving body rubbing (Mann & Smuts 1999). This newborn developmental stage in dolphins compares with the very early young-at-foot stage in kangaroos. The early and repetitive close contact in dolphins likely promotes and reinforces a bond that extends into adulthood, as in many primate species (Silk 2002). In contrast, kangaroo mothers spend more time with their young-at-foot daughters than sons but seem to interact infrequently with their young-at-foot offspring, other than during nursing episodes. As a result, although female kangaroos may be philopatric and settle near close kin as adults, they appear to have few of those early affiliative interactions that are necessary for social bonds to develop. Perhaps more importantly, there may be less advantage for adult kangaroos in forming kin associations than there is for frugivorous or piscivorous species, since kangaroos feed on grass, a dispersed and non-defensible resource. Kinship also plays a limited role in the social system of similar-sized herbivores such as bighorn ewes *Ovis canadensis* (Festa-Bianchet 1991) and mountain goat nannies *Oreamnos americanus* (Festa-Bianchet & Côté 2008).

4.6 ACKNOWLEDGEMENTS

We wish to thank Wilsons Promontory National Park rangers D. Bone, D. Jones and M. Hoskins for logistical support. G. Coulson, M. Festa-Bianchet, U. Gélin and E. Hynes captured and marked females with young. M. Festa-Bianchet provided some observational data. S. Blomberg provided statistical advice. Constructive criticism on preliminary drafts of this manuscript was provided by G. Coulson, M. Festa-Bianchet, D. Fisher and M. Noad. Financial support was received from the Holsworth Wildlife Research Endowment and the Natural Sciences and Engineering Research Council of Canada.

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Table 1. Parameters for fixed effects in the final linear mixed-effects model of proportion time vigilant for 50 eastern grey kangaroo mothers at Wilsons Promontory National Park, Victoria, Australia (N = 365). Values are given as percentages.

	Estimate	SE	t	p
Intercept	4.631	0.046	10.13	0.0001
Cohort (2010/2011 *vs.* 2009/2010)	0.331	0.048	2.64	0.011
Maximum wind speed (in km/h)	0.000032	0.000008	2.01	0.046
Group size	–0.035	0.000	–4.30	0.0001

Table 2. Parameters for fixed effects in the final linear mixed-effects model of mean distance between 50 mothers and 55 offspring in eastern grey kangaroos at Wilsons Promontory National Park, Victoria, Australia (N = 365).

	Estimate	SE	t	P
Intercept	–1.08	1.42	–0.21	0.84
Sex (female)	–1.23	1.09	–2.35	0.022
Age (in months)	1.09	1.02	3.84	0.001
Cohort (2010/2011 *vs.* 2009/2010)	1.31	1.10	2.94	0.005
Time of day (afternoon)	4.02	1.63	2.84	0.005
Maximum wind speed (in km/h)	–1.02	1.00	–3.11	0.002
Age X Time of day (afternoon)	–1.07	1.03	–2.06	0.040

Table 3. Parameters for fixed effects in the final generalised linear mixed-effects model using logistic regression of whether Hinde's proximity index was positive or negative for 50 mother-offspring pairs aged 10.0 to 21.4 months in eastern grey kangaroos at Wilsons Promontory National Park, Victoria, Australia ($N = 312$).

	Estimate	SE	z	P
Intercept	2.68	0.70	3.80	0.001
Age (in months)	–0.12	0.04	–2.76	0.006
Time of day (afternoon)	–0.69	0.25	–2.81	0.005

Table 4. Parameters for fixed effects in the final generalised linear mixed-effects model using logistic regression of whether offspring were seen suckling or not for 112 mother-offspring pairs aged 10.0 to 21.4 months in eastern grey kangaroos at Wilsons Promontory National Park, Victoria, Australia ($N = 201$).

	Estimate	SE	z	P
Intercept	–2.25	0.19	–11.78	0.001
Sex (female)	–0.14	0.16	–0.84	0.40
Age (14–17 months *vs.* 10–13 months)	–0.65	0.18	–3.63	0.001
Age (18–21 months *vs.* 10–13 months)	–2.17	0.35	–6.27	0.001
Cohort (2009/2010 *vs.* 20008/2009)	0.70	0.17	4.13	0.001
Cohort (2010/2011 *vs.* 2008/2009)	0.56	0.21	2.60	0.009
Sex (female) X Age (14–17 months)	0.47	0.25	1.89	0.059
Sex (female) X Age (18–21 months)	1.31	0.42	3.11	0.002

Table 5. Mean number (± 1SE) of mother-offspring interactions and distress calls per day seen for eastern grey kangaroos aged 10–13 months (Period 1), 14–17 months (Period 2) and 18–21 months (Period 3; *N*=107 juveniles) at Wilsons Promontory National Park, Victoria, Australia. 'K-W' refers to Kruskal-Wallis test statistic comparing the three ages. Sample sizes were 71, 76 and 54 juveniles for the three periods, respectively. Superscripts refer to results of Dunn's post-hoc tests comparing pairs of periods, with different letters representing significant differences at $P < 0.05$.

	Age				
	Period 1	Period 2	Period 3	K-W	*P*
Allogrooming	$0.02^{a} \pm 0.00$	$0.02^{a} \pm 0.00$	$0.01^{a} \pm 0.00$	5.88	0.05
Play	$0.02^{a} \pm 0.00$	$0.01^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	12.31	0.002
Nosing	$0.01^{a} \pm 0.00$	$0.01^{a} \pm 0.00$	$0.01^{a} \pm 0.00$	1.47	0.48
Aggressive	$0.00^{a} \pm 0.00$	$0.01^{a} \pm 0.00$	$0.01^{a} \pm 0.00$	5.82	0.05
Distress Call	$0.03^{a} \pm 0.00$	$0.03^{a} \pm 0.01$	$0.01^{b} \pm 0.00$	10.53	0.005

Table 6. Mean number (± 1SE) of mother-offspring interactions and distress calls per day seen for male and female eastern grey kangaroos aged 10–13 months (Period 1), 14–17 months (Period 2) and 18–21 months (Period 3; *N*=107 juveniles) at Wilsons Promontory National Park, Victoria, Australia. '*U*' refers to Mann-Whitney *U* test statistic comparing the sexes. Sample sizes were 39 and 32, 46 and 30, and 33 and 21, for males and females over the 3 periods, respectively.

	Sex			
	Male	Female	*U*	*P*
Allogrooming Period 1	0.01 ± 0.00	0.02 ± 0.01	534.0	0.18
Allogrooming Period 2	0.02 ± 0.01	0.01 ± 0.00	607.5	0.24
Allogrooming Period 3	0.01 ± 0.00	0.01 ± 0.00	342.5	0.90
Play Period 1	0.02 ± 0.01	0.02 ± 0.01	506.0	0.78
Play Period 2	0.01 ± 0.00	0.01 ± 0.00	671.0	0.73
Play Period 3	0.00 ± 0.00	0.00 ± 0.00	334.5	0.65
Distress Call Period 1	0.02 ± 0.01	0.04 ± 0.01	566.5	0.44
Distress Call Period 2	0.02 ± 0.01	0.03 ± 0.01	642.0	0.54
Distress Call Period 3	0.01 ± 0.00	0.01 ± 0.00	338.5	0.82
Nosing Period 1	0.01 ± 0.00	0.01 ± 0.00	571.5	0.39
Nosing Period 2	0.01 ± 0.00	0.01 ± 0.00	659.5	0.63
Nosing Period 3	0.00 ± 0.00	0.01 ± 0.00	299.0	0.13
Aggressive Period 1	0.00 ± 0.00	0.00 ± 0.00	601.5	0.46
Aggressive Period 2	0.00 ± 0.00	0.01 ± 0.01	637.0	0.15
Aggressive Period 3	0.01 ± 0.00	0.01 ± 0.01	300.5	0.19

Table 7. Final model of a linear mixed-effects analysis of HWIs between mothers and offspring for eastern grey kangaroos at Wilsons Promontory National Park, Victoria, Australia, observed between April 2010 and June 2012, $N = 427$.

Coefficient	Estimate	Standard error	*t*	*P*
Intercept	0.701	0.024	29.12	0.001
Sex (female)	0.071	0.037	1.93	0.056
Age (14-17 months)	-0.135	0.027	-4.92	0.001
Age (18-21 months)	-0.448	0.030	-14.77	0.001
Age (22-25 months)	-0.592	0.032	-18.51	0.001
Age (26-29 months)	-0.627	0.038	-16.71	0.001
Age (30-33 months)	-0.607	0.042	-14.54	0.001
Age (34-37 months)	-0.627	0.045	-13.96	0.001
Age (38-41 months)	-0.612	0.065	-9.47	0.001
Age (42-45 months)	-0.678	0.106	-6.41	0.001
Age (46-49 months)	-0.677	0.146	-4.64	0.001
Age (50-53 months)	-0.677	0.146	-4.64	0.001
Sex (female) X Age (14–17 months)	0.094	0.043	2.20	0.029
Sex (female) X Age (18–21 months)	0.138	0.049	2.82	0.005
Sex (female) X Age (22–25 months)	0.086	0.053	1.63	0.10
Sex (female) X Age (26–29 months)	0.032	0.058	0.55	0.58
Sex (female) X Age (30–33 months)	-0.006	0.065	-0.09	0.93
Sex (female) X Age (34–37 months)	-0.031	0.069	-0.45	0.65
Sex (female) X Age (38–41 months)	-0.073	0.086	-0.85	0.40
Sex (female) X Age (42–45 months)	-0.023	0.139	-0.16	0.87
Sex (female) X Age (46–49 months)	-0.034	0.171	-0.20	0.84
Sex (female) X Age (50–53 months)	-0.053	0.206	-0.26	0.80

Figure 1. Distribution of 189 eastern grey kangaroo births at Wilsons Promontory National Park, 2008-2011.

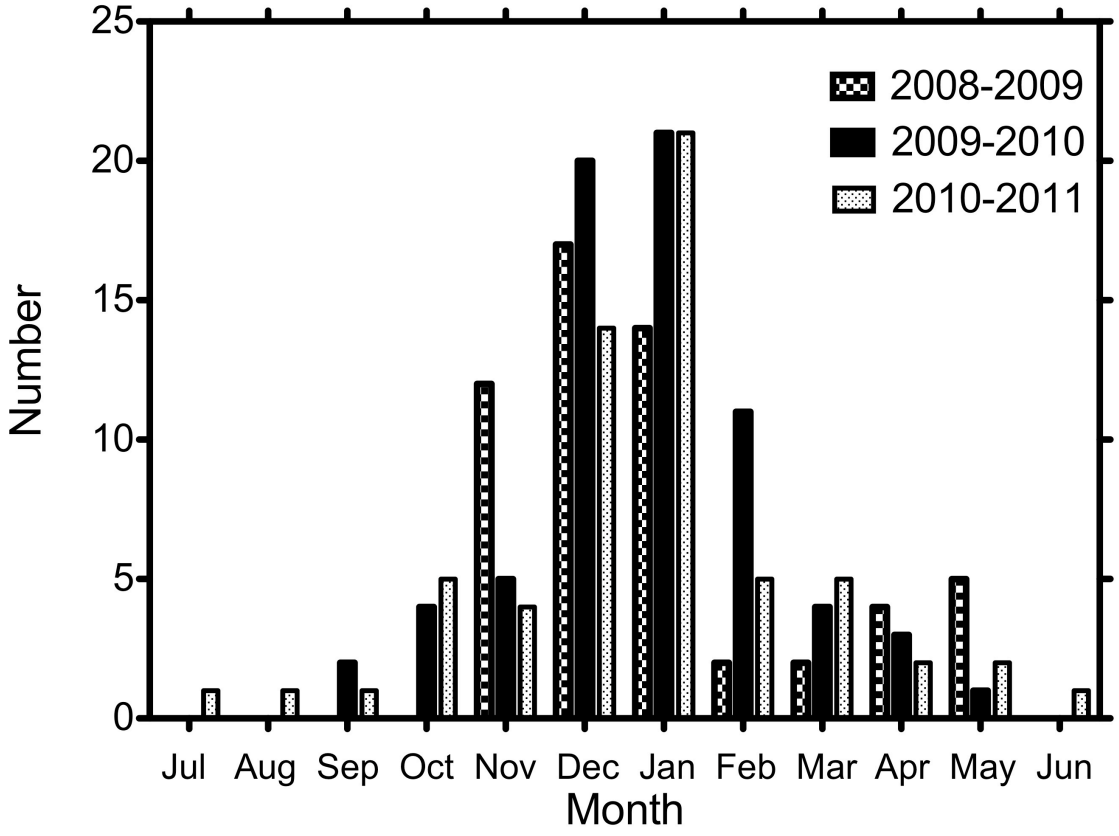


Figure 2. Rates of nursing (± 1SE) between adult female and juvenile eastern grey kangaroos of different sexes and ages at Wilsons Promontory National Park, Victoria, Australia, between April 2010 and June 2012. Period 1 is 10–13 months of age, Period 2 is 14–17 months and Period 3 is 18–21 months. Numbers above bars are sample sizes.

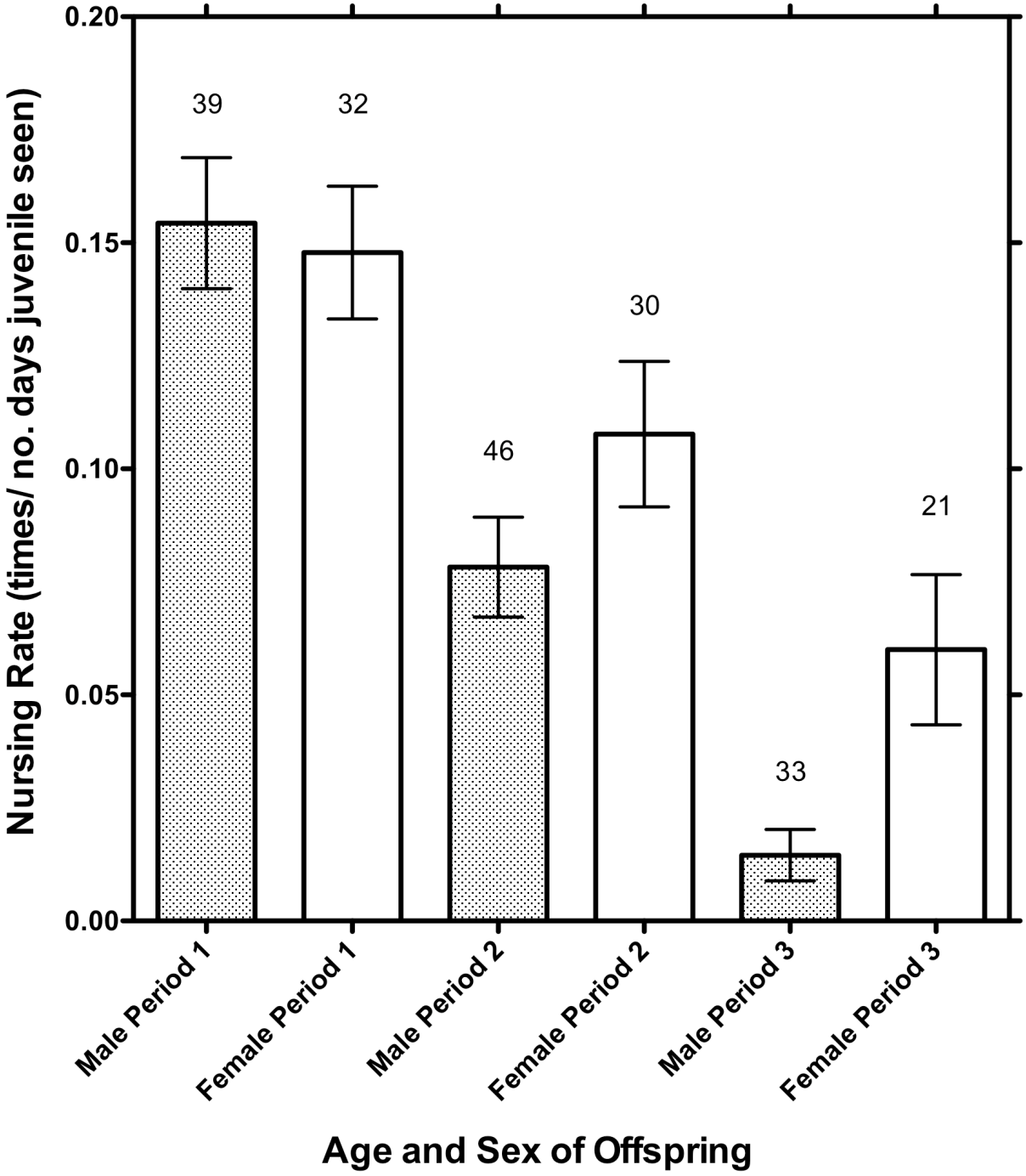
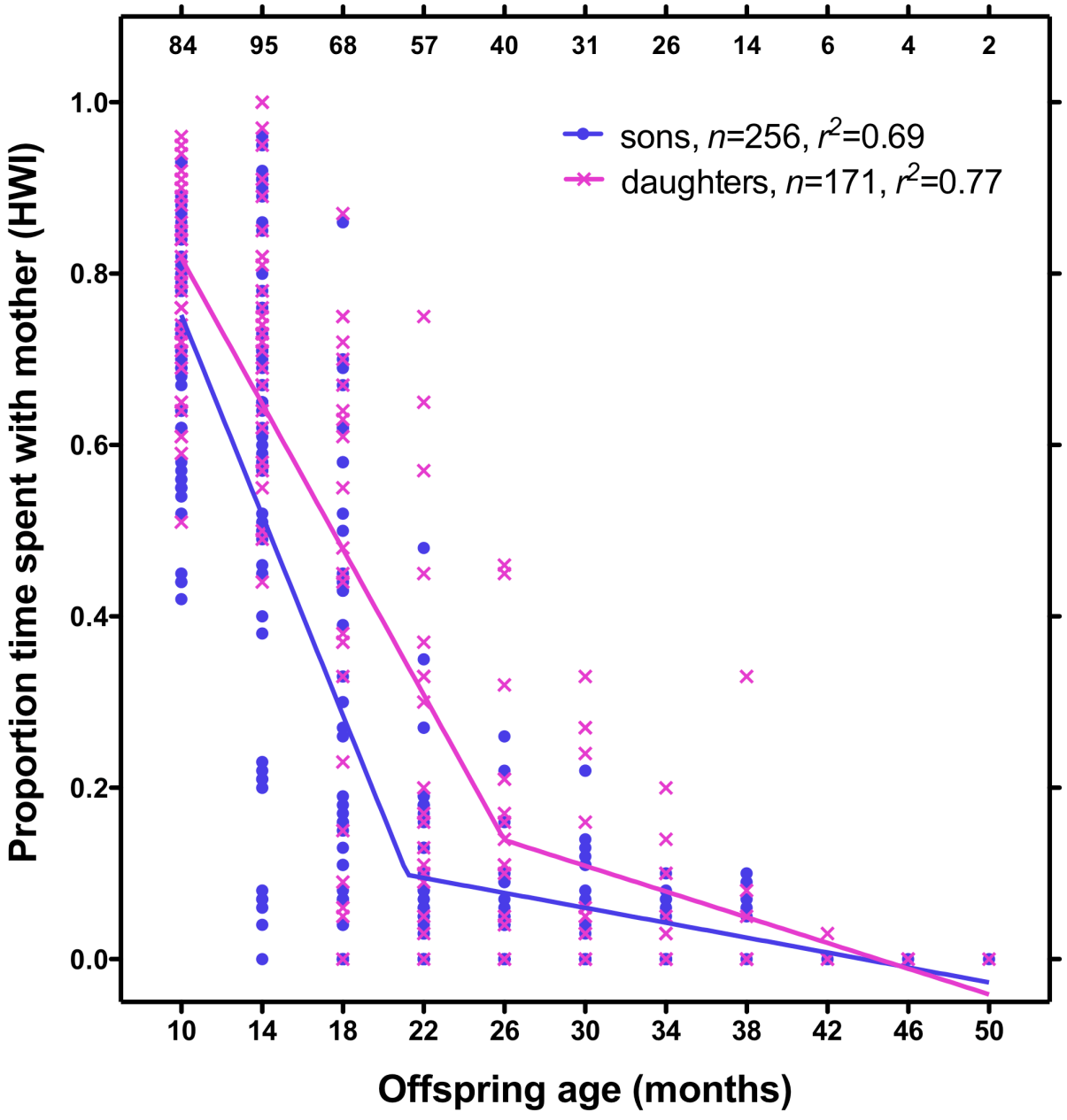


Figure 3. Half-weight indices (HWIs) between 138 eastern grey kangaroos aged 10-53 months and their mothers at Wilsons Promontory National Park, Victoria, Australia. Sons are represented by closed circles and daughters by 'X's. Sample sizes are above the points. The lines indicate segmental linear regression models.



CHAPTER 5: Adoption in eastern grey kangaroos: a consequence of misdirected care?

Wendy J. King, David M. Forsyth, Graeme Coulson & Marco Festa-Bianchet

Manuscript published in *PLoS One* 10:e0125182. This is a revised version.

5.1 ABSTRACT

Adoption is rare in animals and is usually attributed to kin selection. In a 6-year study of eastern grey kangaroos (*Macropus giganteus*), 11 of 326 juveniles were adopted. We detected eight adoptions by observing behavioural associations and nursing between marked mothers and young and three more by analysing the relatedness of mothers and young using microsatellite DNA. Four adoptions involved reciprocal switches and three were by mothers whose own pouch young were known to subsequently disappear. Adoptive mothers were not closely related to each other or to adoptees but adoptive mothers and young associated as closely as did biological pairs, as measured by half-weight indices. Switch mothers did not associate closely. Maternal age and body condition did not influence the likelihood of adoption but females were more likely to adopt in years with high densities of females with large pouch young. Adoption did not improve juvenile survival. We conclude that adoptions in this wild population were potentially costly and likely caused by misdirected care, suggesting that eastern grey kangaroos may have poorly developed mother-offspring recognition mechanisms.

5.2 INTRODUCTION

Adoption, the exclusive care of conspecific non-offspring, is rare in animals [1]. Instances of alloparenting, including nursing, food provisioning, guarding, carrying and grooming, however, have been reported in at least 120 mammalian species across most major orders, including marsupials (in captivity) [1]. Alloparenting has been hypothesised to result from (1) kin selection [2], (2) reciprocal altruism [3], (3) improved parental experience and/or (4) misdirected care [1,4], but allonursing in particular appears to evolve where costs are low [5]. Kin selection involves related females sharing parental duties, including the nursing of each other's young, as often occurs in communally breeding rodents [6] and carnivores [7] but it has also been invoked for adoption in an asocial sciurid [8]. Reciprocal altruism involves unrelated females sharing parental duties: young could gain immunological benefits such as antibodies from allosuckling, potentially increasing their survival [9], but there are no published cases of reciprocal allonursing among

unrelated mammals in the wild [4]. Improved parental experience could occur when inexperienced individuals take on parenting duties, potentially increasing future reproductive success [10]; most studies, however, report that the more experienced females adopt following the loss of an offspring [4]. Misdirected care in the absence of kin benefits occurs in situations where females breed at high density and cannot avoid allonursing, such as in some bats and seals [1,11], or in species with poor offspring recognition, where juveniles may be confused and adopted following a disturbance [12].

Occurrences of adoption of pouch young in wild eastern grey kangaroos (*Macropus giganteus*) led us to investigate the factors responsible for this behaviour. Here we describe behavioural associations between mothers and adopted young in a high-density population of kangaroos and assess the validity of the adoption hypotheses listed above. Eastern grey kangaroos are gregarious herbivores, which live in fission-fusion groups [13]. Adult females do not form close associations with each other [14]. Females usually give birth to a single altricial offspring after a gestation of 36 days [15]. The young develops in the pouch and permanently emerges at about 10.5 months of age, following an 'in-and-out' period of about 5 weeks, with brief initial sorties from the pouch at 6.5 months of age [15]. Following permanent emergence from the pouch, the young is referred to as a young-at-foot and continues to suckle until at least 18 months of age [15]. Reproduction is seasonal, with most young permanently leaving the pouch in October and November in southeastern Australia [16,17].

5.3 MATERIALS AND METHODS

We sampled and measured 326 juvenile and 194 adult female eastern grey kangaroos in Wilsons Promontory National Park, Victoria, Australia, between 2008 and 2013. We marked all captured adults and 316 young in the pouch or at foot [18] and aged them according to body measurements [19]. We classified adult females according to incisor wear as prime-aged or old [20]. Adult females that were both small (arm length $\leq$ 207 cm) and light (mass < 23.5 kg) at first capture were classified as 3-year-olds (young). We evaluated parity based on appearance of the pouch and teats [15]. We estimated body condition as the standardised residual of a linear regression of the logarithm of body mass on hind leg length [21], using different regressions for adult females, male pouch young and female pouch young. We collected tissue samples for genetic analyses from the ear of females and young. We estimated the density of females with large pouch young each winter by multiplying the estimated density of kangaroos in July from distance sampling along fixed transects [22] by the proportion of marked females with large pouch young in August/September. Densities of kangaroos ranged between 5 and 7/ha [23] and we marked approximately one half the adult females present each year. Only about 50% of marked females

successfully raised offspring to the large pouch young stage (approximately 8 months of age) in any year (Table S1). We assessed juvenile survival to 21 months of age. The main predator was the introduced red fox (*Vulpes vulpes*), which can kill young eastern grey kangaroos [24].

We initially detected adoptions through observations of associations, pouch occupation and nursing between marked mothers and young (see Figure 1). To assess the effects of maternal age, body condition and population density on the likelihood of adoption and juvenile survival, we compared those factors for the 11 adoptive females for the year they adopted to all other years they were monitored (range 2–5 years). We fitted generalised linear mixed-effect logistic regressions with binomial errors in R version 2.15.2 [25], using maternal identity as a random factor. We sequentially removed the least significant parameter (based on its *P*-value, threshold $\geq$0.05) from models using stepwise backward selection [26]. Predictors for whether females adopted included age class, body condition, winter density of females with large pouch young and all 2-way interactions. Predictors for juvenile survival also included whether the offspring had been adopted. We did not include parity in analyses because it was correlated with age (all primiparous females were young) and was unknown at first capture. We compared the proportion of adoptive females that successfully raised an offspring to the large pouch young stage in the year following an adoption to the population average using a Fisher Exact Test. Intensive observations (approximately 45 h/month) from April 2010 to June 2012 allowed assessment of mother-offspring associations using half-weight indices (HWIs) calculated in SOCPROG 2.4 [27]. HWIs measure the proportion of time individuals are seen together in the same group [27] and we defined a group using the 10-m chain rule [28]. We compared 4-month HWIs between mothers and adopted young to those between mothers and biological offspring using *t*-tests. We also calculated HWIs between adoptive and biological mothers (if both individuals were known) from intensive observations taken in August through November 2010–2013. We investigated genetic relationships using 9 highly polymorphic microsatellite markers (G12-6, G16-1, G16-2, G19-1, G26-4, G31-1, G31-3, T3-1T and T32-1) [29,30], according to the methods of King et al. [31] and calculated pairwise relatedness (*r*) using KINGROUP v2 [32]. Mothers and putative young with mismatched genotypes without behavioural data on adoption were re-sampled and re-analysed for verification of non-relatedness. Spreadsheets of (1) characteristics of adoptive mothers and offspring survival for all years monitored, (2) genotypes of adoptive mothers and offspring and (3) HWIs of mothers with offspring are available at Dryad Digital Depository, http://dx.doi.org/10.5061/dryad.jr531.

The study was conducted under permits from the Victorian Department of Sustainability and Environment (#1004582, #1005558 and #100007062) and approved by the Animal Ethics Committees of the University of Melbourne (#0810628.1, #0911512.1 and #1312902.1) and the University of Queensland (#SIB/206/09/(NF)).

5.4 RESULTS

We recorded 11 adoptions among a total of 326 juveniles (3%; Table 1). We detected eight adoptions through observations of associations and nursing between marked mothers and young, and another three through DNA analyses (Table 1). Four adoptions involved reciprocal switches between pairs of mothers (#6 with #115 and #64 with #310; Table 2) and four were by mothers whose own pouch young disappeared (#81, #166, #441 and #492; Table 2), of which three had been marked (those of #166, #441 and #492). One mother was seen to reject her offspring after a non-offspring had occupied her pouch, by kicking at it when it approached and attempted to follow her (#81). One mother was captured with two young in her pouch and subsequently abandoned her own offspring (#492; Table 1). Both sexes were adopted and adoptees varied in body condition (mean ± SE: –0.011 ± 0.017; Table 1). Young were adopted when 7–11 months of age and those that survived to permanent pouch emergence were allonursed on average for 6.1 months following adoption (Table 1). All adoptive mothers were already lactating and usually adopted young of the same age and sex as their biological offspring (Table 2). When the difference in estimated age of the two offspring was more than one week, the older and larger offspring was adopted (Table 2). Most adoptive mothers were prime-aged, multiparous and in above-average body condition when captured with a large pouch young, the time when adoptions occurred (Table 2), but the only significant parameter retained in the logistic regression model of the likelihood of adoption was density of females with large pouch young (Table 3; see Figure 2). Most adopted young survived to weaning at around 18 months (Table 1) and 6/11 adoptees survived to at least 59 months of age, including the four involved in reciprocal switches. Adoption did not improve juvenile survival (Table 4). Rather, survival to 21 months increased with maternal body condition and density of females with large pouch young (Table 4). Adopting did not reduce future reproduction because 82% of 11 females produced large pouch young the following year (Table 2) compared to the population average of 53% for females successfully raising offspring to large pouch young stage (Fisher Exact Test, $P = 0.36$). Twelve young were orphaned when 12–17 months of age during our 6-year study. None was adopted before or allonursed after permanent pouch exit, and adult females were generally aggressive towards them. Four of the 12 orphans died within a month (mean age at orphaning was 16 months), while the rest survived for at least 6 months. Maternal care is not necessary for survival at this stage, particularly as the four youngest orphans aged 12 or 13 months all survived.

Mean pairwise relatedness r between 321 mothers and biological offspring was 0.48 ± 0.01 and pairs matched at all 9 loci. Adoptive mothers and young were not closely related (mean $r = -0.03 \pm 0.05$), with mismatches at 2–7 of 9 loci (Table 1). When genotypes of both biological and adoptive mothers were known ($n = 6$), they were not closely related (mean $r = 0.03 \pm 0.02$). The six

pairs of adoptive mothers and young studied in 2010–2012 associated as closely as did biological mother-young pairs. Adopted young showed a non-significant trend for greater association indices at 14–17 months compared to biological pairs (sons: mean HWI = 0.80 ± 0.07, $n = 4$ *vs.* 0.55 ± 0.04, $n = 53$ $t_{55} = 1.85$, $P = 0.07$; daughters: mean HWI = 0.87 ± 0.13, $n = 2$ *vs.* 0.72 ± 0.02, $n = 33$ $t_{33} = 1.46$, $P = 0.15$). No differences occurred at 18–21 months ($t < 0.67$, $P > 0.50$) or 22–25 months ($t < 0.69$, $P > 0.49$). The three known pairs of adoptive and biological mothers did not associate closely in August–November, when adoptions occurred (mean HWI = 0.07 ± 0.06).

5.5 DISCUSSION

Marsupial reproduction, with extended lactation of young in the pouch, has long been exploited in captivity for experimental manipulation and cross-fostering, especially for conservation of rare macropodids [33,34]. Reproductive females readily accept non-offspring pouch young of roughly the same age as that removed, even from other species [34,35]. However, our observations of pouch young adoption are the first in wild kangaroos.

Kin selection does not explain adoptions in kangaroos because females did not adopt closely related young; *r*-values between mothers and adopted young were on average close to zero. Communal rearing of mammalian young by related mothers seems to be key for allonursing to be maintained by kin selection [4] but kinship plays little role in the social structure of female eastern grey kangaroos [14].

Most reciprocal exchanges of parental care in unrelated mammals take the form of babysitting [1], but in eastern grey kangaroos the switch was permanent until weaning. We recorded two pairs of females that switched offspring, which is the first documented evidence for mutual cooperation involving reciprocal allonursing among unrelated wild mammals. Reciprocity theory requires equal input from both parents because altruism is open to cheating [36]. Where pouch young were switched, all four mothers exclusively nursed the adopted young for at least 6 months and ceased care of their biological offspring. The reward for mutual cooperation in switching pouch young could be improved offspring survival due to immunological benefits [9]. Unlike eutherian mammals, marsupials receive maternal antibodies over a prolonged period throughout pouch life [37], so adopted young could obtain additional immune compounds from their adoptive mother, increasing survival. However, we did not detect improved juvenile survival in adopted young. Additional observations are required to assess survival benefits of reciprocal switches. Both reciprocal switches that we recorded occurred in years of high density, when there were many mothers with large pouch young and mothers were in good body condition. It is thus possible that in-and-out pouch young are participating in a kind of musical chairs game: if a

lactating mother leaves a group without being followed by her pouch young (and the young does soon not find her), the young needs to quickly find another lactating female with a vacant pouch, or it will die of exposure overnight. Dependent offspring that have not permanently emerged from the pouch follow their mothers closely and give distress calls when lost [38]. Because we had marked three of the four biological young that disappeared after their mother adopted another young, we would have detected if another female had adopted them. Thus only about one half of adoptions involved reciprocity. Also, since individual mothers spent very little time together, it is unlikely that adoptive females were monitoring the behaviour of females with their biological offspring. It is thus more likely that the reciprocal switches resulted from mistaken identity (see below) rather than mutual cooperation.

Adoption was unlikely to improve parental experience because only one of the 11 adoptive mothers was young and primiparous. Although all other adoptive mothers were prime-aged and most were in above-average body condition, these variables did not affect the likelihood of adoption in eastern grey kangaroos. In contrast, adoptive northern elephant seal (*Mirounga angustirostris*) mothers were typically young and inexperienced [10].

The hypothesis of misdirected care to explain adoption is contingent upon poor mother-offspring recognition and/or there being little cost to adoption compared to the costs of detecting, recognising and rejecting non-offspring [4]. Kangaroos appear to recognise offspring through olfaction [38], as do most mammals [39]. Because known orphans were never adopted, we speculate that adoptions may have occurred during a disturbance, with females accepting non-offspring into the pouch without verifying their identity before fleeing. Normally, female kangaroos nose juveniles before allowing them to enter the pouch and are aggressive towards any non-offspring that approach [38]. Taking the time to identify offspring in a disturbance would elevate the risk of mother and young becoming separated, with potentially lethal effects on the young. Once inside the pouch, the adopted young may take on the female's odour and be difficult to distinguish from biological offspring. As for the cost of allonursing from pouch emergence to weaning, adoptions occurred at a time when milk production would soon be declining [40] and adoptive females did not appear to have reduced reproduction the following year. Detection of a cost to reproduction is difficult, however, without manipulation of reproductive effort [41]. Adoption in kangaroos thus appeared to incur few costs once young were permanently emerged from the pouch, but when adoptions were not reciprocal (43% of cases), the adoptive mother's own offspring died, indicating an unusually high cost [5]. That females were more likely to adopt in years with many females with large pouch young also supports the hypothesis of misdirected care, since accidental adoptions are more likely to occur under high-density breeding conditions [1].

Density positively affects the probability of allonursing in Hawaiian monk seals (*Monachus schauinslandi*), another species showing misdirected parental care [42].

The likelihood of accidental allonursing or sneaky allosuckling must be lower in pouched marsupials than in eutherian mammals because early lactation takes place exclusively within a pouch. In eastern grey kangaroos, the neonate develops and suckles within the mother's pouch for approximately 10 months before permanent emergence. It is thus only after the young has emerged from the pouch that it could attempt to suckle from another mother. However, because adoptions occurred at the in-and-out stage of pouch young development, an error in maternal recognition of offspring would be more likely to become permanent in marsupials than eutherian mammals if olfactory identity cues are overridden by the offspring being held in the pouch. Also, because offspring develop in a pouch, there should be no selective pressure for offspring recognition before pouch emergence.

Simultaneous adoption of a second offspring into the pouch in wild monotocous marsupials is extremely rare but has been reported in a high-density urban population of common brushtail possum (*Trichosurus vulpecula*) [43]. Eymann et al. [43] suggested that kin selection was responsible for this adoption because the individuals shared mitochondrial DNA haplotypes, but relatedness was not estimated. Biological twins have occasionally been observed in several macropodids, including eastern grey kangaroos, however one twin always dies in the pouch at a young age [15,44,45].

Robust examples of adoption are rare in wild mammals. A 0.2% frequency of adoption in red squirrels (*Tamiasciurus hudsonicus*) was ascribed to kin selection [8]. Adoption in our 6-year study of wild eastern grey kangaroos was an order of magnitude more frequent, at 3%. Since (1) adoptive kangaroo mothers were not closely related to each other or to the adoptees, (2) reciprocal switches did not always occur, (3) switch mothers did not associate closely, (4) most adoptive mothers were not young and inexperienced, (5) adoption did not improve offspring survival, (6) females were more likely to adopt at high density and (7) adoptive mothers and young associated as closely as did biological pairs, we conclude that adoption was likely caused by misdirected care. Our results suggest that mother-offspring recognition mechanisms may be poorly developed in eastern grey kangaroos. The relatively high frequency of adoptive behaviour observed in this population indicates that maladaptive behaviour with possible highly detrimental fitness consequences sometimes occurs in natural populations.

5.6 ACKNOWLEDGEMENTS

We thank U. Gélin, C. Le Gall-Payne and E. Rioux-Paquette for field observations of females with adopted young. J. Chambers, H. Presseault-Gauvin and E. Rioux-Paquette performed the genotyping. Comments by M. Crowther, A. Goldizen and two anonymous reviewers improved the manuscript. Funding was received from NSERC of Canada, the Holsworth Wildlife Research Endowment and the Senate Research Committee of Bishop's University.

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Table 1: Characteristics of adopted pouch young (PY) eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2008–2013. Mother A is the putative biological mother and Mother B is the adoptive mother. 'N/A' refers to not available.

ID	Sex	Cohort	*r* with Mother A	*r* with Mother B (no. mismatched loci)	Type evidence[1]	ID PY replaced	Body condition	Age when detected (months)	Age last suckling (months)
89	female	2009	0.35	–0.08 (3)	pouch	116	–0.058	11.2	N/A
116	female	2009	0.44	0.05 (4)	assoc.	89	–0.021	11.0	16.8
132	male	2009	N/A	–0.11 (3)	DNA	N/A	0.009	9.2	N/A
202	male	2010	0.47	0.26 (2)	pouch	307	–0.026	10.9	16.1
307	male	2010	0.60	0.08 (3)	assoc.	202	N/A	11.0	15.2
321	male	2010	N/A	–0.22 (5)	pouch	239[2]	N/A	10.2	18.6
346	male	2010	N/A	–0.32 (7)	DNA	N/A	N/A	15.4[3]	21.4
466	female	2011	N/A	–0.10 (4)	DNA	unmarked	0.094	8.4	8.9[4]
531	male	2012	0.45	0.17 (2)	pouch	unmarked	–0.029	8.6	15.7[5]
615	female	2013	0.50	–0.12 (4)	pouch	622[2]	–0.052	9.9	10.7[4]
620	male	2013	N/A	0.08 (3)	pouch	584[6]	–0.003	7.3[7]	9.6[4]

[1] pouch = seen in a second pouch; assoc. = seen suckling a second mother; DNA = seen with one mother but genotype of young did not match mother.

[2] Subsequently disappeared and presumably died.

[3] Not individually recognisable until marked at 15.4 months of age.

[4] Disappeared at this age and presumably died.

[5] Disappeared at this age and was later found dead.

[6] Abandoned soon after capture.

[7] Found in the pouch with another young (#584) that was alone in the pouch when first captured at 1.4 months of age.

Table 2: Characteristics of adoptive female eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2008–2013. 'N/A' refers to not available.

ID	Sex adopted	Cohort	ID other mother	Sex replaced	Age difference in young (d)	Body condition	Reproductive status	Reproductive success following year
115	female	2009	6	same	+6	+0.064	N/A	weaned son
6	female	2009	115	same	–6	+0.015	multiparous	weaned daughter
131	male	2009	N/A	N/A	N/A	+0.086	N/A	produced daughter[1]
310	male	2010	64	same	–1	+0.024	N/A	weaned daughter
64	male	2010	310	same	+1	+0.027	multiparous	weaned son
166	male	2010	N/A	same	–2	+0.046	multiparous	weaned daughter
303	male	2010	N/A	N/A	N/A	+0.022	N/A	lost small pouch young
349	female	2011	N/A	different	+13	+0.010	primiparous	weaned daughter
81	male	2012	363	same	N/A	N/A	multiparous	lost small pouch young
441	female	2013	36	same	+26	–0.027	multiparous	produced daughter[2]
492	male	2013	N/A	same	+9	–0.019	N/A	lost large pouch son

[1] Disappeared at 17.1 months of age and presumably died.

[2] Still alive as a young-at-foot at 12 months of age in January 2015.

Table 3. Parameter estimates for fixed effects retained in the generalised linear mixed-effects logistic regression model of whether or not 11 adult female eastern grey kangaroos adopted a pouch young at Wilsons Promontory National Park, Australia, 2009–2013 (n = 43).

	Estimate	SE	z	P
Intercept	–3.37	0.99	–3.41	0.001
Density of females with LPY[1]	0.84	0.32	2.61	0.009

[1] large pouch young

Table 4. Parameter estimates for fixed effects retained in the generalised linear mixed-effects logistic regression model of offspring survival to 21 months for 11 adult female eastern grey kangaroos at Wilsons Promontory National Park, Australia, 2009–2013 (n = 43).

	Estimate	SE	z	P
Intercept	–6.46	2.38	–2.72	0.006
Maternal body condition	305.4	122.0	2.50	0.012
Density of females with LPY[1]	2.23	0.88	2.52	0.012
Maternal body condition X Density of females with LPY[1]	-88.8	37.4	-2.37	0.018

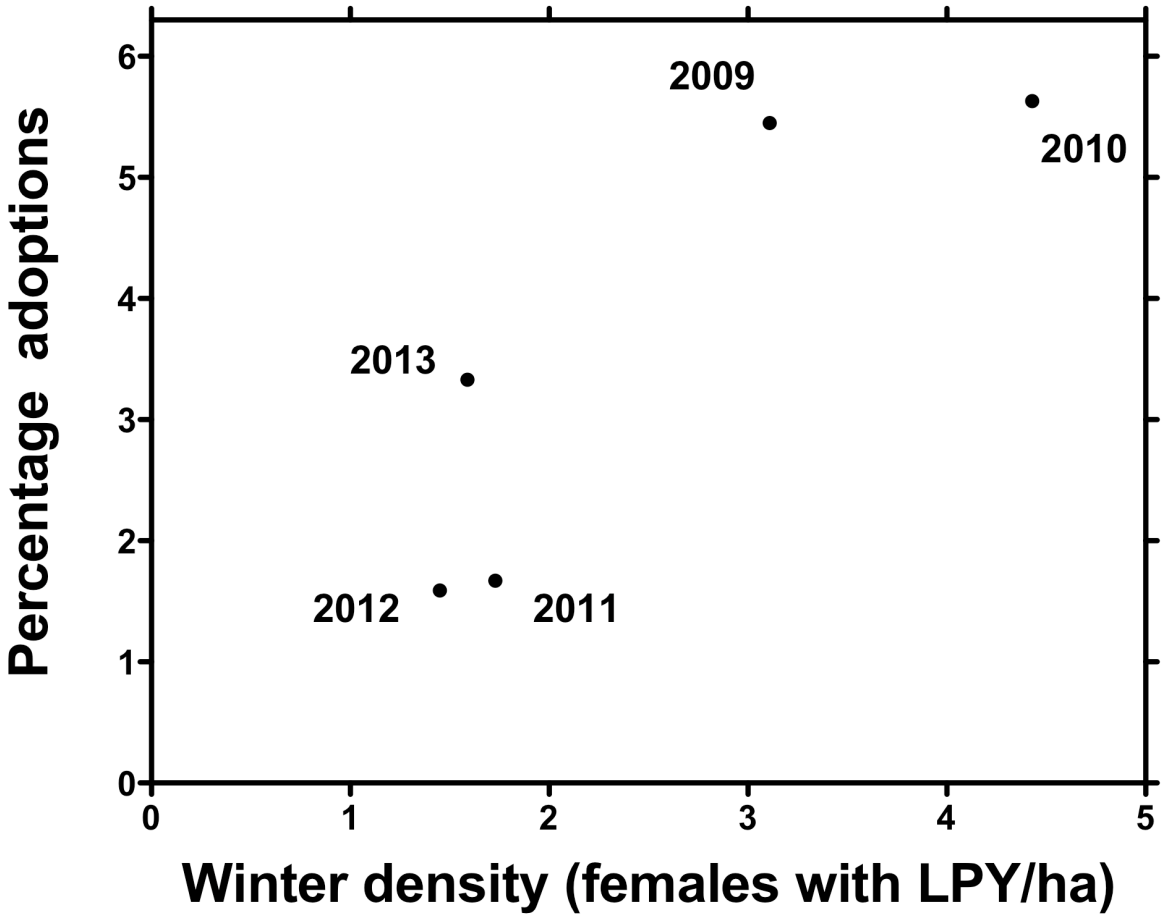
[1] large pouch young

Table S1. Numbers of marked adult female kangaroos raising an offspring to large pouch stage (approximately 8 months of age) each year from 2008 to 2013 at Wilsons Promontory National Park, Australia.

Year	Successful	Unsuccessful	Total	Success rate (%)
2008	17	25	42	40
2009	59	16	75	79
2010	71	22	93	76
2011	56	76	132	42
2012	64	69	133	48
2013	62	81	143	43
Total	329	289	618	53

Figure 1. Female #81 with adopted young #531 in her pouch. The light blue eartag in the ear of #531 was applied when captured in the pouch of female #363. Photo courtesy of C. Le Gall-Payne.

Figure 2. Annual percentage adoptions as a function of density of female eastern grey kangaroos with large pouch young (LPY) in winter at Wilsons Promontory National Park, Australia. Density was not estimated in 2008 before the study started.



CHAPTER 6: Benefits of mother-offspring associations in eastern grey kangaroos

Wendy J. King, Marco Festa-Bianchet, Graeme Coulson & Anne W. Goldizen
Manuscript to be submitted to *Behavioral Ecology and Sociobiology*.

6.1 ABSTRACT

Social relationships can have adaptive value and maternal care should enhance offspring survival and growth. Eastern grey kangaroos *Macropus giganteus* are gregarious and females are weakly philopatric. Adult females do not form strong bonds with other females but associate closely with their juvenile offspring. We aimed to determine whether the strength of mother-offspring associations correlated with survival, growth and reproduction of offspring. We observed 129 offspring aged 10 to 21 months with their mothers in a high-density population at Wilsons Promontory National Park, Australia. We marked young in the pouch or as young-at-foot. We used half-weight indices to quantify associations between mothers and their juvenile offspring and determined the proportion of time juveniles spent alone with their mother while foraging. Association indices and time spent alone with the mother were not correlated with juvenile survival, body condition as 2-year-olds or reproduction as 3-year-olds. However juveniles that spent a large proportion of time with their mothers at 18–21 months were 6% larger and 18% heavier as 2-year-olds than those that did not associate with their mothers. Stronger mother-offspring associations before weaning therefore had a beneficial effect on growth of juveniles, which likely reflects maternal care.

6.2 INTRODUCTION

Environmental conditions experienced during early development affect survival, growth and reproduction in vertebrates (Lindström 1999). Parents contribute both genetic material and environmental influences to their offspring and one important factor in early development of mammals is maternal care (Clutton-Brock 1991). Care of the young broadly encompasses nursing, food provisioning, guarding, carrying and grooming of the young, all of which may contribute to the offspring's survival and phenotype (Maestripieri and Mateo 2009). Juveniles that receive high-quality care may experience improved survival and growth in vertebrates such as spotted hyenas *Crocuta crocuta* (Hofer and East 1993), savannah baboons *Papio cynocephalus* (Altmann and

Alberts 2005) and bighorn sheep *Ovis canadensis* (Festa- Bianchet et al. 2000). Although maternal effects in mammals are largely assumed to be nutritional, other maternal effects are possible such as maternal defense and manipulation of the nest environment (Bernardo 1996). In species where milk transfer or food provisioning does not occur, maternal defense may increase offspring fitness *e.g.*, skinks *Egernia whitii* (Sinn et al. 2008). Maternal defense can indirectly affect offspring growth by reducing conspecific harassment of offspring, which allows the young more time to feed (Clutton-Brock 1991). Environmental conditions that mothers experience influence the resources available for reproduction (Monaghan 2008).

Females in stable societies can form long-term associations that improve reproductive success (Silk 2007). Social bonds established through spending time in close proximity and allogrooming often increase dominance and priority of access to food (Silk 2007) and may reduce glucocorticoid stress hormones (Silk et al. 2012), which likely translate through maternal effects into improved offspring survival (Silk et al. 2003). In species with unstable grouping patterns, associations between group members are temporary but could nonetheless be beneficial (Frère et al. 2010).

Eastern grey kangaroos *Macropus giganteus* live in fission-fusion groups that appear to form as an anti-predator strategy (Jarman and Coulson 1989). Females can produce a single offspring annually and live up to two decades (Poole 1982). Offspring are nursed for about 18 months, the first 10 months of which are spent in the pouch (Poole 1983). Although females are gregarious, they show some tendency to isolate themselves from larger groups when accompanied by an offspring that has permanently emerged from the pouch but is still suckling (Chapter 2). Females in foraging groups may form weak preferential associations with other females (Best et al. 2014), the benefits of which are unknown.

We aimed to determine whether the strength of the relationship between mothers and their offspring affects fitness in a long-lived marsupial. We attempted to elucidate the relationship between maternal care and offspring fitness in a species with strong fission-fusion dynamics. We thus evaluated the effect of sociability on growth, survival and reproduction of offspring in eastern grey kangaroos. Social integration is often assessed as a composite measure based on proximity to adult conspecifics, approaches to others and allogrooming rates (Silk et al. 2003; Cameron et al. 2009). Because adult female kangaroos rarely interact with their offspring except to nurse them (Chapter 4), we estimated sociability as 1) association indices between mothers and offspring based on foraging group memberships and 2) the proportion of time offspring spent alone with the mother during active foraging periods. We predicted that these social factors would be positively correlated with growth, survival and reproduction of offspring.

6.3 METHODS

We studied eastern grey kangaroos in Wilsons Promontory National Park, Victoria, Australia (38° 57' S, 146° 17' E) from April 2010 to December 2012. Kangaroos inhabit a 110-ha study area that consists of meadows surrounding a grassy landing strip. The area is mostly open with occasional trees and bushes such as coast tea-tree *Leptospermum laevigatum*, coast wattle *Acacia longifolia* and coast banksia *Banksia integrifolia* (Davis et al. 2008). There were no wild dogs but predators on juveniles included red foxes *Vulpes vulpes* and wedge-tailed eagles *Aquila audax*.

Captures to mark pouch young and assess reproductive status of female kangaroos commenced in 2008 and occurred annually. We marked adult kangaroos with plastic eartags and collars, and pouch young with small plastic eartags for visual identification (King et al. 2011). Whether a female produced a pouch young and/or was nursing a young-at-foot was established at her capture through examination of the condition of her pouch and state of the teats (Poole 1975; Poole 1983). Loss or weaning of an earlier offspring was established through presence of a regressing teat (Poole 1983). Captured animals were weighed and measured. Body condition was estimated as the standardised residual of the linear regression of the logarithm of body mass on hind leg length (Schulte-Hostedde et al. 2005), calculating separate regressions for male pouch young, female pouch young, other males and other females. Although there are other methods for measuring body condition of widely differing sizes of animals (Peig and Green 2010), our measure was used to estimate the relative body condition of animals that were captured at about the same age and size, *e.g.*, adult females or pouch young. The birthdates of young kangaroos were calculated as the mean of estimates from hind leg, foot and head lengths, using different curves for males and females (Poole et al. 1984). We confirmed mother-offspring relationships using nine highly polymorphic microsatellite markers isolated from ear tissue samples (Chapter 5). There were seven cases of adoption over the three cohorts, but because there was no apparent difference between associations of mothers with the two types of young (Chapter 5), we treated adopted young as biological young.

We observed kangaroos for 2–3 h after dawn and before dusk using 8X32 binoculars (Leitz, Germany). Observations involved one observer (WJK) walking slowly around sections of the study area for 10 to 12 consecutive days each month between April 2010 and June 2012 (total 1,122 h). A second observer (MFB) took additional observations from July 2010 to August 2011, in October 2011 and in March 2012, providing approximately 25% of the observations. We recorded group size (including unmarked individuals) and group composition of marked individuals.

Behavioural observations began in 2010 when most juveniles in the first study cohort (2008/2009) were about 15 months of age. These young were weaned at approximately 18 months

of age, and several months later, a second cohort (2009/2010) emerged from the pouch at around 10 months of age (Poole 1975), and a third cohort (2010/2011) emerged one year later. Behavioural observations ended in June 2012 when the third cohort was 17 months of age, on average. Young that have permanently emerged from the pouch but continue to suckle are termed young-at-foot. Sometimes suckling continued to 23 months of age if the mother did not reproduce successfully the next year, but suckling events of older offspring may be difficult to detect (Chapter 4). We examined the behaviour of young-at-foot until 21 months of age and defined sub-adults as those that were 22–30 months old, *i.e.*, 2-year-olds.

Survival of juveniles was monitored monthly from June 2010 to December 2012. The study area was searched for approximately 10 consecutive days per month and animals were noted as disappearing on the date last seen. Six marked juveniles from the first study cohort disappeared before June 2010, when the study area had only been monitored every other month, and so their age at disappearance was not known to the nearest month; three were 8–11 months old, two were 11–14 months old and one was 14–16 months old. These animals were not included in analyses. Juveniles in the third cohort were at least 20 months old in December 2012. The youngest known disperser was 23 months old (Chapter 3), so we assumed all disappearances were mortalities before that age.

We caught and marked 142 pouch young between 22 August 2009 and 14 October 2011, when they were aged 8.4 ± 0.1 months, then recaptured 57 of those individuals approximately 18 months later as 2-year-olds (32 sons and 25 daughters) at 26.3 ± 0.2 months of age, between 7 March 2011 and 7 March 2013. Offspring ages were estimated to 0.1 month, using birthdates calculated from sex-specific curves in Poole et al. (1982). Because young were born throughout the year and the time between captures varied, we estimated growth as size (hind leg length) and mass at recapture, taking age at recapture into account (range 22.0–29.9 months). We also estimated body condition of 2-year-olds. We did not assess growth for 16 other offspring caught and marked as young-at-foot because they were first captured when much older (mean 15.1 ± 0.6 months) than pouch young, yielding a less accurate estimate of birthdate (Poole et al. 1982). Natural mortality of young peaked at 10 months and again at 18 months of age, and most mortality (96%) occurred before the age of 21 months (Figure 1), so we chose to assess the factors correlated with the survival of 139 young from 3 cohorts to the age of 21 months. Thirteen daughters in the first cohort survived to 3 years of age and their reproduction in their fourth year of life was assessed as follows: no pouch young (coded as 0), produced a pouch young and lost it (coded as 1), or carried a pouch young to permanent emergence (coded as 2). Whether a female produced a pouch young and lost it was assessed at annual capture by examination of the teats. None of the eight surviving daughters in the second cohort produced a pouch young in their fourth year of life.

We measured two aspects of mother-offspring sociability. First we assessed how much time offspring spent in foraging groups with their mothers, using the half-weight index (HWI) in SOCPROG (Whitehead 2008) for 4-month periods from April 2010 to June 2012. Groups were defined using the 10-m chain rule (Jarman 1987). We also estimated the proportion of time offspring spent alone with their mothers while actively foraging over the same time periods. These values were calculated for 129 individual offspring that were potentially suckling as young-at-foot and aged 10–13, 14–17 and 18–21 months, if we had at least 10 observations for both the mother and the young within these periods. We correlated HWIs with proportions of time young spent alone with the mother using Pearson correlations. Because observations spanned three cohorts of young over two years, not all pairs were observed in all periods (Table 1). No juveniles from the third cohort were observed at 18–21 months of age and only two of the 3-year-old females from the first cohort were observed at 10–13 months. Since sample sizes were uneven across offspring ages, we conducted analyses on social factors separately for each age period.

We evaluated the effects of age and sex on the proportion of time young spent alone with the mother using linear mixed-effects models, treating offspring identity as a random factor, and age, sex and cohort as fixed effects. Survival of offspring to 21 months of age was analysed using generalised linear logistic regression, with offspring sex, cohort, offspring body condition, maternal body condition, HWI at 10–13 months and time spent alone with the mother at 10–13 months as fixed factors. We did not examine the effect of social factors at later ages because a large proportion of mortality (61%) had occurred by 17 months of age (Figure 1). Factors affecting size of the hind leg, mass and body condition at 2 years of age were assessed using linear models and analysis of covariance (ANCOVA) and the same fixed factors as for survival, plus age at recapture, and HWIs and time spent alone with the mother at 14–17 months and 18–21 months. Reproduction of thirteen 3-year-old daughters from the first cohort was compared with HWIs and time spent alone with the mother at 14–17 months and 18–21 months using Spearman rank correlations, due to small sample sizes. Linear models were run in R version 2.15.2 (R Development Core Team 2012). We sequentially removed the least significant parameter (based on its P-value, threshold ≥ 0.05) from the model using stepwise backward selection (Crawley 2007). Initial models included interactions between offspring age and sex, and between cohort and maternal condition. Where the social factors were not retained in the final model, we present the z-, t- and P-values for those factors from the complete model including all fixed effects.

Captures were undertaken with ethics approval from the University of Melbourne (#0810628.1 and #0911512.1) and research permits from the Victorian Department of Sustainability and Environment (#1004582 and #1005558). Observations were conducted with animal ethics approval from the University of Queensland (#SIB/206/09/(NF)).

6.4 RESULTS

The time that young spent in groups with the mother (HWI index) was positively correlated with the time that they spent alone with her, although the relationship was weakest at 10–13 months (Pearson correlation at 10–13 months: $r = 0.24$, $P = 0.024$, $n = 84$; at 14–17 months: $r = 0.64$, $P = 0.001$, $n = 91$; at 18-21 months: $r = 0.82$, P = 0.001, $n = 73$). Time spent alone with the mother decreased as the offspring aged and was less for sons than daughters, and in the first cohort (Table 2, Figure 2). Individual identity contributed 16% to the overall variance in time spent alone with the mother.

Measures of mother-young sociability when young were 10–13 and 14–17 months did not affect leg size of 2-year-olds ($|t| < 0.13$, $P > 0.90$, $n = 28$ and $|t| < 0.45$, $P > 0.65$, $n = 47$), but time spent in groups with the mother (HWI) at 18–21 months had a positive influence on leg size, as did age of recapture and cohort ($F_{4,42} = 12.1$, $P = 0.001$, adjusted $R^2 = 0.49$, $n = 47$; Table 3). Sub-adults were larger when male, older and in the first cohort. Mean leg length of sub-adults was 447 ± 3 mm and an increase in HWI from 0.00 to 0.87 resulted in a 6% increase in leg length.

Mass was also not influenced by aspects of sociability at 10–13 or 14–17 months ($|t| < 0.53$, $P > 0.60$, $n = 28$ or $|t| < 1.10$, $P > 0.28$, $n = 47$). Time spent in groups with the mother (HWI) at 18–21 months, however, had a positive effect on mass, together with sex, age of recapture, cohort and maternal body condition ($F_{5,41} = 11.45$, $P = 0.001$, adjusted $R^2 = 0.53$, $n = 47$; Table 4). Mean mass of sub-adults was 17.7 ± 0.4 kg and an increase in HWI from 0.00 to 0.87 resulted in an 18% increase in mass. An increase in maternal body condition from –0.072 to +0.107 resulted in a 27% increase in mass of sub-adults at 2 years of age. Maternal body condition was not correlated with the time young spent in groups with the mother (HWI) at any age (Pearson correlations; $0.11 > r > -0.08$, $0.99 > P > 0.36$, $90 > n > 72$).

Body condition of 2-year-olds was not related to mother-young sociability at any previous age (at 10-13 months: $|t| < 1.50$, $P > 0.15$, $n = 28$; at 14–17 months: $|t| < 1.94$, $P > 0.06$, $n = 47$; and at 18–21 months: $|t| < 1.91$, $P > 0.06$, $n = 47$), but was related to cohort, the mother's body condition and the offspring's body condition as a pouch young ($F_{6,48} = 10.3$, $P = 0.001$, adjusted $R^2 = 0.51$, $n = 55$; Table 5). There was also an interaction between cohort and the mother's body condition: maternal body condition had a positive effect on body condition of 2-year-olds in the first and third cohorts but not the second (Table 5). Mean body condition of 2-year-olds was –0.005 ± 0.003 and decreased by 0.014 from cohort 1 to cohort 2 and by 0.026 from cohort 1 to cohort 3. As maternal body condition increased from –0.072 to +0.107, condition of 2-year-olds increased by 0.082. As pouch young body condition increased from –0.125 to +0.139, condition of 2-year-olds

increased by 0.066. Although body condition of pouch young was positively correlated with body condition of the mother at capture (Pearson correlation, $r = 0.37$, $P = 0.001$, $n = 112$), both factors contributed significantly to the selected model of condition of 2-year-olds (Table 5). Mothers of the third cohort had much lower body condition than mothers in the first two cohorts (Figure 3).

Survival to 21 months was not related to mother-young sociability at 10–13 months ($|z| < 1.72$, $P > 0.06$, $n = 74$.) Survival was strongly influenced by cohort ($|z| > 2.95$, $P < 0.004$, $n = 113$; Table 6). For young with data on sociability, however, the final model only included body condition when first caught as a large pouch young ($z = 2.27$, $P = 0.023$, $n = 74$; Figure 4). There were few observations at 10–13 months for cohort 1 (Table 1), for which survival from permanent pouch emergence to 21 months of age was high (96%, $n = 53$; see also Figure 1). Survival was 57% for cohort 2 (n = 56) and 23% for cohort 3 ($n = 30$).

Reproduction of 3-year-old daughters was not correlated with sociability measures (Spearman rank correlations at 14–17 months, $n = 12$, time spent in groups with the mother (HWI): $P = 0.27$, $r_s = –0.35$; time spent alone with the mother: $P = 0.22$, $r_s = –0.39$; at 18–21months, $n = 13$, time spent in groups with the mother (HWI): $P = 0.30$, $r_s = 0.31$; time spent alone with the mother: $P = 0.16$, $r_s = 0.41$), despite considerable ranges in HWIs (0–1.00 and 0–0.72, respectively) and time spent alone (0–0.44 and 0–0.27, respectively).

6.5 DISCUSSION

Growth of the young was mostly related to non-social factors. There were large differences in size, mass and body condition of 2-year-olds depending on cohort. Body condition and mass of 2-year-olds was affected by their condition as pouch young and by the body condition of their mother. These body-condition measures likely reflect non-social maternal effects on growth of young (Mousseau and Fox 1998; Maestripieri 2009). Body condition of mothers when they are carrying a large pouch young is likely affected by available resources (Lindström 1999). In this species, lactation costs appear to peak when females are carrying a large pouch young (Cripps et al. 2011), after which young emerge from the pouch and begin to feed on forage. Body condition of the large pouch young likely reflects maternal care up to that point. The condition of both mother and large pouch young at capture seemed to influence body condition of the offspring as a 2-year-old.

Mother-offspring sociability measures had no discernable relationship with offspring survival in kangaroos. Survival of offspring to 21 months was weakly influenced by body condition as pouch young. Stochastic factors such as predation, inclement weather or poor forage availability probably have a much stronger effect on survival, as suggested by the strong cohort

effects. In a 'good' year, almost all juveniles survived whereas in years of higher mortality, body condition as a pouch young had a positive effect on survival. We propose that in the absence of stochastic environmental factors, maternal characteristics determine offspring survival. Similar results have been found for allied rock-wallabies *Petrogale assimilis* (Delean et al. 2009).

Social factors probably had a minor effect on growth of young, after the maternal effects outlined above. Indeed, sub-adult mass was more strongly influenced by maternal body condition than by time spent with the mother (HWI). We have shown elsewhere that kangaroo mothers do not have a protective parenting style and dependent offspring maintain the relationship with the mother by following her (Chapter 4). Juveniles that spent a lot of time with their mothers between 18–21 months of age were most likely still suckling, obtaining nutrition from their mothers as well as feeding on forage. Only the time spent in foraging groups with the mother (not the time spent alone with the mother while actively foraging) at this age was correlated with sub-adult leg size and mass, so the association index likely reflects maternal care. Mothers and their dependent offspring do not cooperate to defend resources since forage is uniformly distributed and no large macropods are territorial (Jarman and Coulson 1989), although there is some evidence for low-level interference competition (Jaremovic and Croft 1991; Maguire et al. 2006). The effect of time spent with the mother on subsequent size and mass thus likely reflects direct care through nursing rather than a bond that is maintained through frequent interactions and allogrooming.

We report elsewhere that mothers with young-at-foot tended to isolate themselves from other adult females (Chapter 2), so there is likely a cost to associating closely with others in kangaroos. Female macropods may isolate themselves with their dependent offspring for several reasons. Croft (1981) suggested that mother red kangaroos *Macropus rufus* may have restricted movements due to the reduced locomotor ability of their young-at-foot and so may not be able to maintain associations with other adults. He also proposed that mothers isolate themselves to prevent the young from getting separated from the mother when sudden disturbance causes flight. This second hypothesis has also been put forward for eastern grey kangaroos (Southwell 1984; Jarman 1994). Separation from the mother sometimes had lethal effects for pouch young at the in-and-out stage in this study (Chapter 5). Similarly, mothers may isolate themselves to teach young escape routes. Fleeing juveniles that have been disturbed in a large group appear to follow all sex-age classes indiscriminately and sub-adults often do not flee in a direct manner (WJ King, unpublished data). Also, it is possible that close associations between non-juveniles increase competition for forage, as reported for red-necked wallabies *Macropus rufogiseus* (Johnson 1986). Alternatively, mothers may isolate themselves to reduce the harassment of young-at-foot by other adult females, which takes the form of growling, aggressive displacements and batting and occurs most often when young are foraging in groups without their mother (Chapter 4). Mothers spent an

average of 35% of their time isolated with young-at-foot (Chapter 2) and so spent most of their time in larger groups. In addition, according to our definition, being isolated meant that they were at least 10 m away from other kangaroos (Chapter 2), thus anti-predator advantages could still have been obtained if other kangaroos were within sight. Kangaroos do appear to form groups due to predator pressure because vigilance rates of mothers decreased with group size (Chapter 4) and fox predation seems to reduce juvenile survival (Banks et al. 2000). It is possible that foxes were taking advantage of juveniles' poor body condition since fox presence was relatively high (Chapter 4) and body condition was relatively low in the year with poorest juvenile survival. These two factors could thus be working synergistically on offspring survival.

Time spent with the mother as a young-at-foot was not correlated with reproduction of 3-year-old daughters, however sample sizes were small. Furthermore, these data came from a cohort that had experienced very high survival and growth and so social effects may have been masked. We found cohort effects in all measures of growth and survival. Cohort also affected reproduction since none of the eight 3-year-old daughters from the second cohort reproduced, compared to 46% of 13 daughters in the first cohort. Cohort effects on offspring growth and survival have long been recognised in mammals, especially ungulates (Lindström 1999). Marsupial reproduction appears to be adapted to unpredictable environmental conditions (Tyndale-Biscoe and Renfree 1987). To distinguish between maternal environmental effects and genetic influences on offspring phenotype, Lindström (1999) suggested cross-fostering experiments, which generally are more feasible in birds than viviparous mammals. Alternatively, maternal effects can be estimated using the animal model and pedigrees (Wilson et al. 2009). Recently, McAdam et al (2014) suggested the combination of cross-fostering experiments within a pedigree analysis to partition maternal variance into genetic and environment components. With the accumulation of at least three generations of pedigree data, one could also incorporate the animal model (Wilson et al. 2009). Because marsupials give birth to altricial young and many macropod species readily accept pouch young from other females (Merchant and Sharman 1966; Robert et al. 2010), they are ideal for testing the relative importance of maternal effects during early development.

6.6 ACKNOWLEDGEMENTS

Funding was received from the Natural Sciences and Research Council of Canada and the Holsworth Wildlife Research Endowment. C. Le Gall-Payne and R. Glass helped monitor the population in 2012. S. Blomberg provided statistical advice. D. A. Fisher and M. Noad commented on previous versions of this manuscript.

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Table 1. Sample sizes of 129 juvenile eastern grey kangaroos observed in three different cohorts from April 2010 to June 2012 at Wilsons Promontory National Park, Australia.

	Cohort		
Age	2008/2009	2009/2010	2010/2011
10–13 months	3	53	29
14–17 months	34	51	11
18–21 months	40	35	0
Overall	44	55	30

Table 2. Final model of a linear mixed-effects analysis of proportion time offspring spent alone with their mothers while actively foraging for eastern grey kangaroos at Wilsons Promontory National Park, Australia, observed between April 2010 and June 2012, n = 244.

Coefficient	Estimate	Standard error	t	P
Intercept	0.227	0.024	9.29	0.001
Sex (female)	0.063	0.018	3.54	0.001
Age (14–17 months *vs.* 10–13 months)	–0.081	0.019	–4.25	0.001
Age (18–21 months *vs.* 10–13 months)	–0.178	0.022	–8.18	0.001
Cohort (2009/2010 *vs.* 2008/2009)	0.064	0.021	3.05	0.003
Cohort (2010/2011 *vs.* 2008/2009)	0.063	0.030	2.07	0.041

Table 3. Final model of ANCOVA for size of the hind leg (in mm) of sub-adult eastern grey kangaroos first captured as large pouch young and then recaptured approximately 18 months later at Wilsons Promontory National Park, Australia, between August 2009 and March 2013, $n = 47$. HWI = half-weight index.

Coefficient	Estimate	Standard error	t	P
Intercept	289.2	43.0	6.73	0.001
Sex (female)	–28.5	5.5	–5.15	0.001
Age at recapture (in months)	6.5	1.6	4.00	0.001
Cohort (2009/2010 *vs.* 2008/2009)	–18.5	5.5	–3.35	0.002
HWI with mother at 18–21 months	29.79	10.9	2.73	0.009

Table 4. Final model of ANCOVA for mass (in kg) of sub-adult eastern grey kangaroos first captured as large pouch young and then recaptured approximately 18 months later at Wilsons Promontory National Park, Australia, between August 2009 and March 2013, $n = 44$. HWI = half-weight index.

Coefficient	Estimate	Standard error	*t*	*P*
Intercept	3.11	5.14	0.60	0.55
Sex (female)	–3.17	0.65	–4.86	0.001
Age at recapture (in months)	0.59	0.19	3.08	0.004
Cohort (2009/2010 *vs.* 2008/2009)	–2.97	0.65	–4.56	0.001
Body condition of the mother	26.88	8.48	3.17	0.003
HWI with mother at 18–21 months	3.70	1.28	2.88	0.006

Table 5. Final model of ANCOVA for body condition of sub-adult eastern grey kangaroos first captured as large pouch young and then recaptured approximately 18 months later at Wilsons Promontory National Park, Australia, between August 2009 and March 2013, $n = 55$.

Coefficient	Estimate	Standard error	t	P
Intercept	–0.009	0.004	–2.25	0.029
Cohort (2009/2010 *vs.* 2008/2009)	–0.014	0.008	–1.77	0.08
Cohort (2010/2011 *vs.* 2008/2009)	–0.026	0.012	–2.20	0.033
Body condition of young	0.252	0.071	3.56	0.001
Body condition of the mother	0.460	0.110	4.18	0.001
Cohort 2009/2010 X Body condition of the mother	–0.465	0.187	–2.48	0.016
Cohort 2010/2011 X Body condition of the mother	–0.290	0.204	–1.42	0.16

Table 6. Final model of generalised mixed-effects logistic regression for survival to 21 months of eastern grey kangaroos first captured as large pouch young at Wilsons Promontory National Park, Australia, between August 2009 and December 2011, $n = 113$.

Coefficient	Estimate	Standard error	z	P
Intercept	3.61	1.01	3.56	0.001
Cohort (2009/2010 *vs.* 2008/2009)	–3.13	1.06	–2.96	0.003
Cohort (2010/2011 *vs.* 2008/2009)	–4.91	1.11	–4.41	0.001

Figure 1. Deaths or disappearances of juvenile eastern grey kangaroos (n = 80) in 3 cohorts of juveniles at Wilsons Promontory National Park, Australia, that were marked from August 2009 to December 2011 at approximately 8 months of age and followed to December 2012. Cohort 1 = 2008/2009, Cohort 2 = 2009/2010, Cohort 3 = 2010/2011.

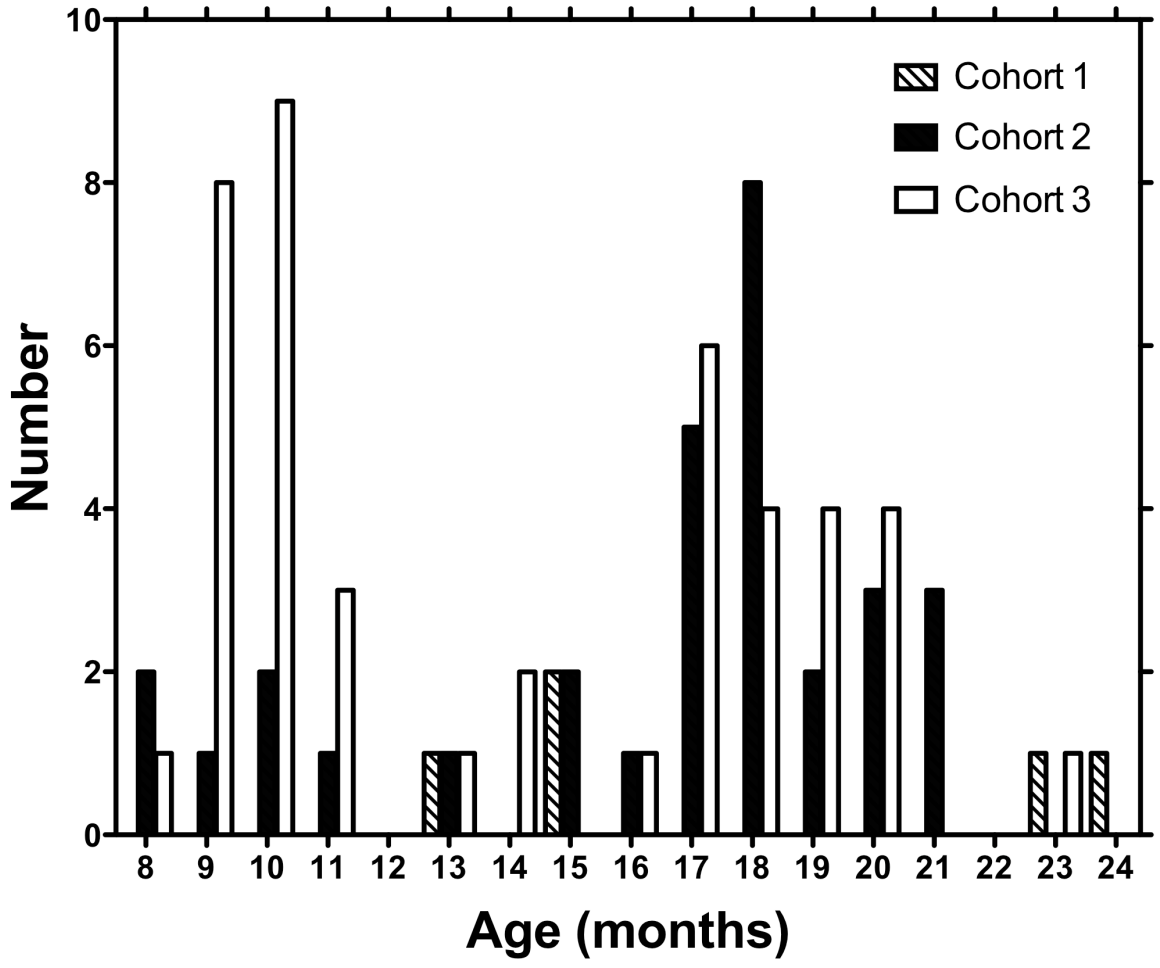


Figure 2. Mean proportion of time (± 1SE) sons and daughters spent alone with their eastern kangaroo grey mother while actively foraging at ages 10–13 months, 14–17 months and 18–21 months between April 2010 and June 2012 at Wilsons Promontory National Park, Australia. Numbers above bars are sample sizes.

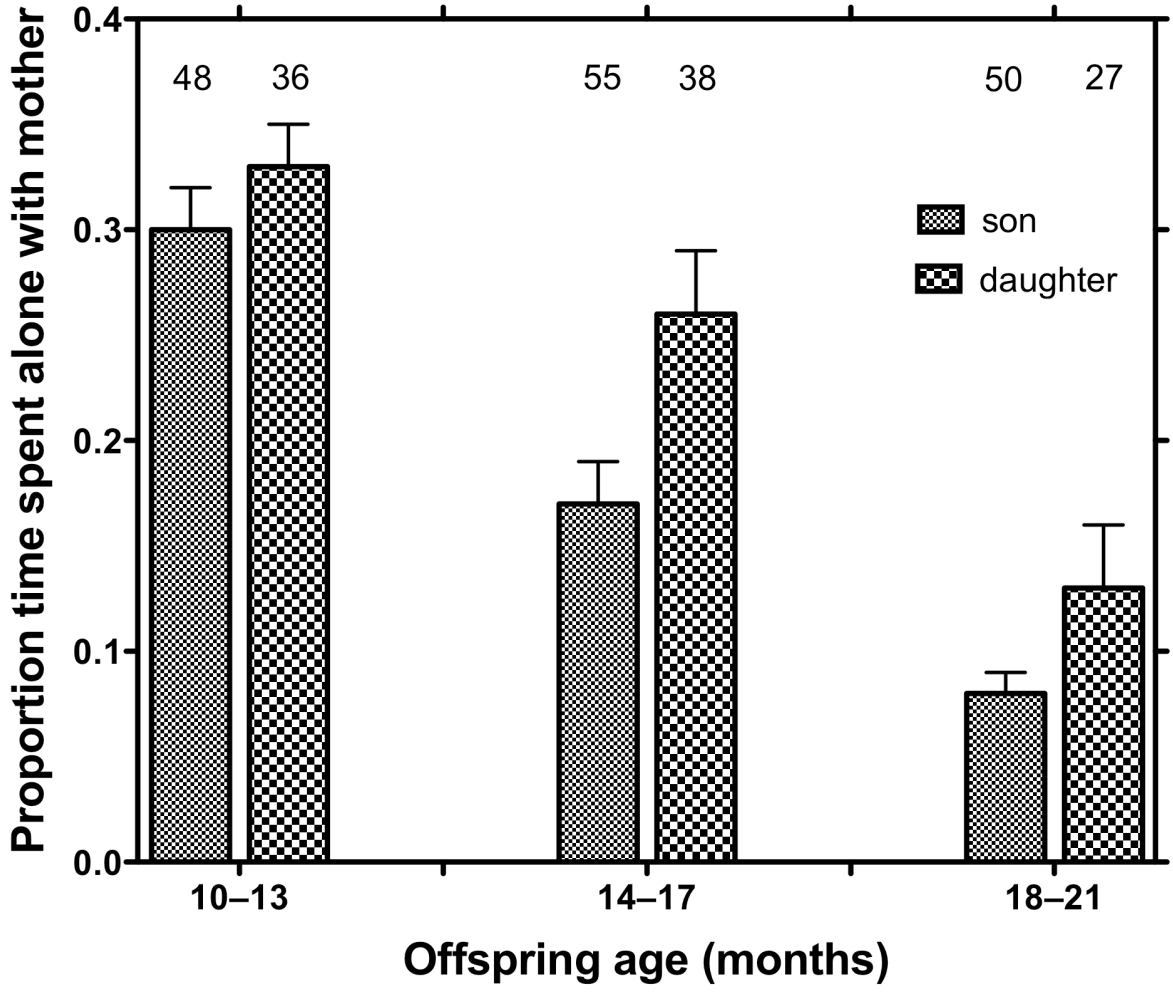


Figure 3. Body condition (± 1SE) of female eastern grey kangaroos and their large pouch young captured at Wilsons Promontory National Park, Australia, 2009-2010 (*n* = 113 pairs). Cohorts were 2008/2009, 2009/2010 and 2010/2011.

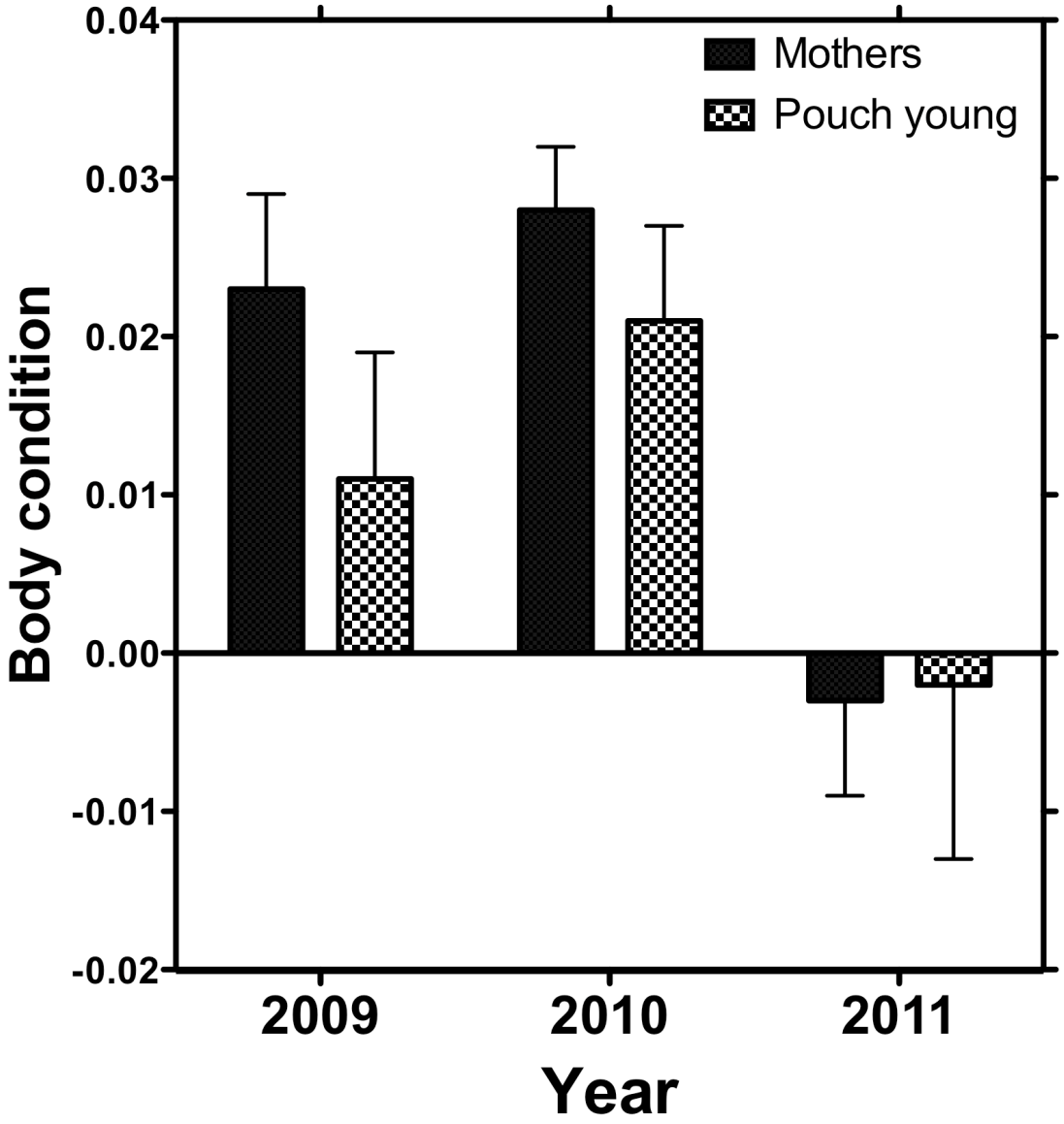
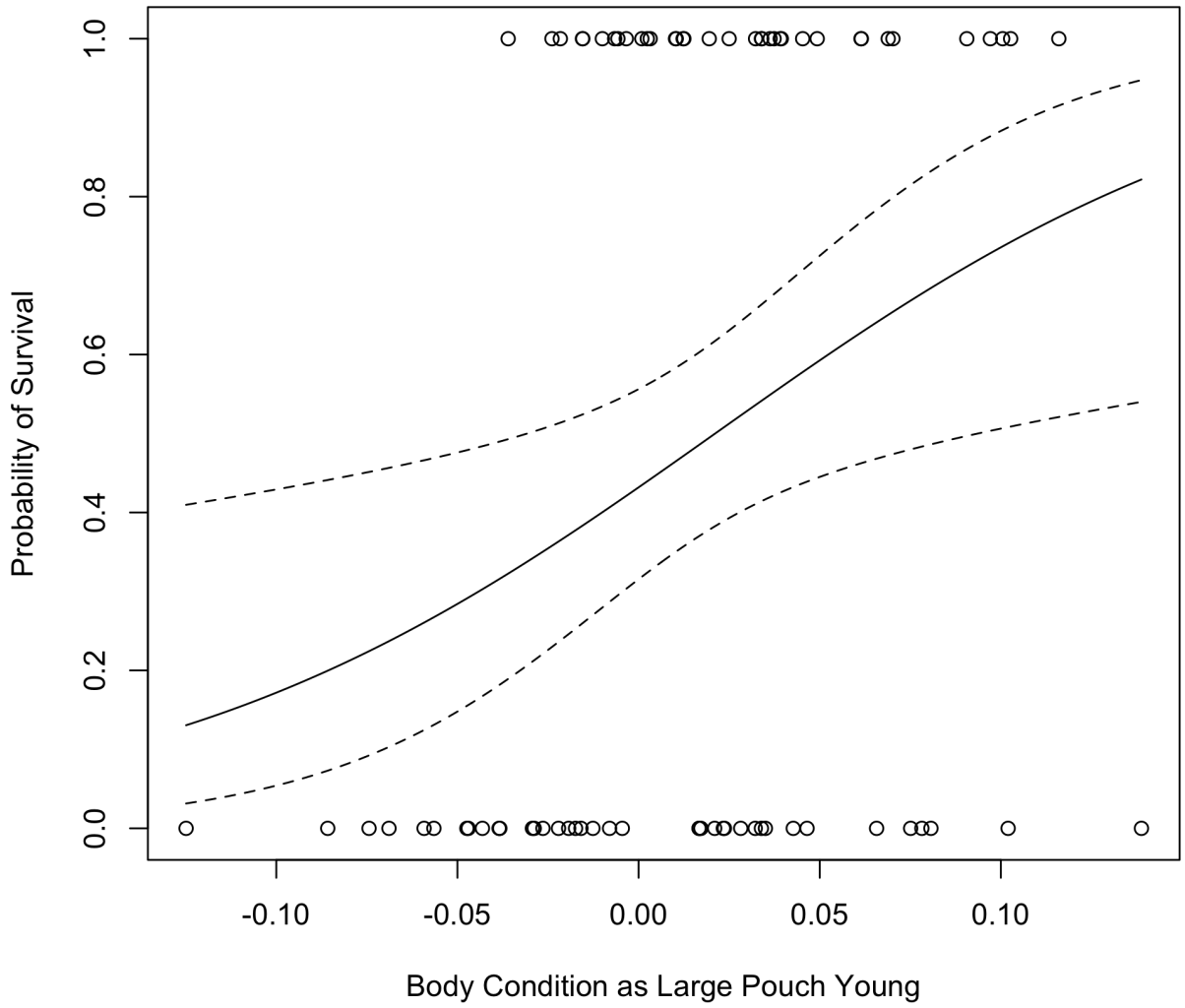


Figure 4. Generalised linear logistic regression of body condition as a large pouch young on survival to 21 months for eastern grey kangaroos first captured at Wilsons Promontory National Park, Australia, between August 2009 and December 2011, $n = 75$. The solid line represents predicted values and the dotted lines are 95% confidence intervals.



CHAPTER 7: General Conclusion

7.1 SUMMARY

I have shown in this thesis that weak but non-random associations occurred among individuals in this high-density population (Chapter 2). Weak associations among adult females resulted from philopatry but were not based on kinship because density decreased mean relatedness among neighbouring females (Chapter 3). Strong associations occurred between adult females and their young-at-foot (Chapter 4) and the strength of mother-offspring associations had a positive effect on juvenile growth (Chapter 6). The benefits of these associations, however, were likely a direct result of greater maternal care through nursing rather than reduced predation or increased access to resources.

One factor that could influence the complexity of social relationships is the ability to recognise other individuals through advanced cognition (Dunbar 1992; Dunbar and Schultz 2007). It has been hypothesised that mammals do not form persistent associations with other individuals unless there has been simultaneous development of the neocortex (Dunbar 1992; Dunbar and Schultz 2007). This encephalisation has occurred in several mammalian suborders such as Hippomorpha, Anthropoidea, Strepsirrhine, Odontoceti, Caniformes and Tylopoda (Schultz and Dunbar 2010) but not in large macropods (Ashwell 2008). It thus appears that animals such as eastern grey kangaroos that rely on olfactory cues and neuroendocrine priming to establish mother-offspring bonds (rather than visual cues and endogenous opioids) may be unable to extend these relationships into adulthood (Broad et al. 2006). Increases in relative brain size across ungulates, carnivores and primates appear to have evolved secondarily as a response to sociality (Pérez-Barbería et al. 2007).

Earlier (Chapter 1), I described routes to stable *vs.* unstable grouping patterns that were based on ecological conditions and considered that predation and the distribution and predictability of resources are the main driving factors of whether animals form temporary or long-lasting relationships (Gowans et al. 2008). Aureli et al. (2008) postulate that there are two routes from asociality to higher fission-fusion dynamics in animals. The first (A) involves extended associations (such as mother-offspring pairs or mates) becoming advantageous, which leads to cohesive groups. Later, ecological conditions may cause grouping to be costly so that groups must fission. In the second scenario (B), there are no advantages to extended associations but animals come together due to ecological conditions such as predator pressure or a preferred resource and live in fission-fusion groups. These groups may later become more cohesive if ecological

conditions relax. One would then expect the fission-fusion societies from the scenario A to be characterised by greater social complexity and higher communication skills than those fitting scenario B (Aureli et al. 2008). The critical distinction between the two routes to sociality is whether extended associations confer fitness benefits. The two routes I described earlier (Chapter 1, Figure 1) are similar to scenarios A and B in Figure 3 of Aureli et al. (2008), except I suggested that ecological constraints were the primary factors driving grouping patterns and that extended associations developed secondarily. I have been unable to show extended associations between mother-offspring pairs or between mates in kangaroos and thus no potential benefits to these relationships as adults. It thus seems that kangaroos at high density form fission-fusion groups according to scenario B of Aureli et al. (2008), where ecological constraints drive grouping patterns. Although I did not investigate the distribution and predictability of resources, predation risk appears to be a cause of aggregation for adult females, because vigilance rates decreased with group size (Chapter 4).

The social system of eastern grey kangaroos is unlike that of many ungulates of similar size because territories, leks, harems and all-male groups do not occur (Jarman and Southwell 1986). Also, females do not form the same type of matrilines with dominance hierarchies found in cervids (Chapters 2 and 4). Instead, all sex-age classes come together in relatively small groups of 2 to 5 individuals with open membership, despite living in open habitats (Jarman and Southwell 1986). This situation appears most similar to that found for some odontocetes such as northern bottlenose whales *Hyperoodon ampullatus* or bottlenose dolphins *Tursiops aduncus*, although strong male-male associations occur in both those species and likely function in mate acquisition (Connor et al. 1992; Gowans et al. 2001). Jarman and Southwell (1986) hypothesised that the lack of male territoriality by male eastern grey kangaroos was somehow related to their mode of indeterminate growth but they did not explain what could be the relationship. Male kangaroos continue to grow throughout their lifetimes, which should aid in contests to move up in the dominance hierarchy and gain access to females in oestrus (Jarman 1989). Indeterminate growth, which occurs in both male and female kangaroos (M Festa-Bianchet, personal communication), however, is extremely rare in mammals (Sebens 1987). Why kangaroos should continue to invest in growth long beyond reproductive maturity is a question for future research.

Fission-fusion dynamics can be described along the three temporal dimensions of group size, cohesion and composition (Aureli et al. 2008). Because group size appears to be set by ecological constraints as in Figure 1, Chapter 1 (Chapman et al. 1995; Johnson et al. 2002), it is important that the definition of what consists a 'group' be biologically meaningful. Many authors use a set chain rule (Jarman 1987) to distinguish inter-group distances, however, these distances may vary for the same species under different circumstances, such as varying densities. I believe

that the inter-group measure of 10 m that I used reflected the dispersion of kangaroo groups in my study at high density because I first examined the distribution of nearest-neighbour distances. Aureli et al. (2012) proposed new models incorporating both ecological and social factors to determine the critical inter-individual distance between groups, which should aid in the quantification of fission-fusion dynamics.

Not all animals were marked and thus individually recognisable in this study. The monthly proportion of unmarked animals seen in groups with marked individuals ranged between 29 and 43% in the first year of observations and between 25 and 33% the second year. I thus believe the associations I describe here among the 413 marked individuals seen between April 2010 and June 2012 were representative of the population as a whole.

The analyses of mother-offspring associations and space use (Chapter 4) would have been improved by inclusion of more known adult pairs. In my study, this was limited by the time it takes for daughters to reach reproductive maturity (3 years) and the restriction I had for fieldwork. The juvenile cohort that had been marked in 2008 was about 3.5 years old and potentially primiparous when I terminated observations in June 2012. Because the study is being continued by colleagues interested in male reproductive success, offspring sex ratio and timing of birthdates, it may be possible to better evaluate adult mother-offspring relationships in the future. Due to poor juvenile survival rates in recent years, however, sample sizes may still be low. The cohort from 2009 experienced moderate survival (and very poor reproduction in 2013; none produced a pouch young, WJ King, unpublished data) while the cohort from 2010 experienced very poor survival to 2014 (Chapter 6).

7.2 FUTURE DIRECTIONS

I found no strong social structure in this high-density kangaroo population (Chapter 2). This study should thus be repeated at low and/or intermediate densities to determine whether adult females preferentially associate with kin at lower densities. At the same time, spatial autocorrelations should be performed to evaluate the effect of density on female genetic structure in kangaroos. The weak autocorrelation results (Chapter 3) were possibly due to negative density-dependence, so there should be stronger genetic spatial structure at lower density (Busch et al. 2009). My results regarding a lack of close associations among adult female kin may have differed from those of Stuart-Dick (1987) and Jarman (1994) because they used matrilineal relationships rather than bi-parental relatedness coefficients to establish kinship. However, high relatedness coefficient values of around 0.5 among females must indicate mother-daughter or full sister pairs (confirmed in Chapter 5 for mothers and offspring), and mother-daughter pairs are the kin

relationships most likely to be important in philopatric mammalian societies (Michener 1983). I found no close associations between adult females with pairwise relatedness coefficients above 0.4 (Chapter 2), even though adult female kin were available (Chapter 3), so no mother-daughter pairs were associating closely. The behavioural difference between the two studies is thus more likely due to density and should be tested.

While mother-daughter associations were stronger than mother-son associations at early ages, these bonds did not persist into adulthood (Chapter 4). Because associations occurred at low levels and there were few overt interactions among adults, I suggest that we should shift our research focus from trying to elucidate the benefits of associations to evaluating the costs of sociality. Future studies of fission-fusion dynamics of kangaroos could quantify approach rates and nearest-neighbour distances, as used for feral goats *Capra hircus* (Stanley and Dunbar 2013) and spider monkeys *Ateles geoffroyi* (Ramos-Fernández and Morales 2014). One could then answer such questions as: Do individuals tend to leave large groups and join small groups (to reduce feeding competition)? Is there an optimal foraging group size? Does group composition matter or do animals randomly join groups of foraging individuals? I have shown that there were weak associations between different sex-age classes, *e.g.*, adult males spent time with receptive adult females in the breeding season and adult females tended to isolate themselves with their young-at-foot (Chapter 2), so group composition likely is important in group formation and dissolution.

I was unable to investigate associations while animals were resting, due to the difficulty of making observations at those times (Chapter 2). Mammalian behavioural associations can be remotely tracked using proximity data loggers attached to collars (Swain and Bishop-Hurley 2007), which are particularly useful for species with cryptic or highly infectious individuals (Hamede et al. 2009). It is unlikely, however, that these collars could be attached to kangaroo pouch young and so mother-offspring relationships could not be determined from a young age using this method. Future studies of kangaroo social behaviour should attempt to delimit preferred social partners at other times than while actively foraging, and then compare active patterns to resting patterns.

I found that there were benefits to mother-offspring associations in terms of growth of the young (Chapter 6). It would be interesting to determine the mechanism by which spending time with the mother increases sub-adult size and mass. Do young-at-foot that associate closely with their mothers benefit by receiving more milk than other young? As discussed earlier (Chapter 4), quantification of milk transfer is logistically problematic (Cameron 1998) and would need to be undertaken in captivity, which could alter maternal spacing behaviour. Do young-at-foot avoid harassment from other adult females by associating closely with their mothers, which could affect stress levels? This question could be addressed by observing mother-offspring pairs and noting

whether aggression towards young occurs when the young is far from the mother. One could then correlate faecal cortisol levels (Touma and Palme 2005) with observations of aggression.

Another avenue that deserves further research is mother-offspring recognition in kangaroos. Females rarely responded to their own offspring's distress calls (Chapter 4), despite individually distinct vocal signatures (Baker and Croft 1993). Offspring recognition could thus be tested using playbacks of juveniles' calls. Females also may not visually recognise young offspring because mothers sometimes accepted non-offspring into their pouch (Chapter 5). Infrequent separation does not require maternal recognition (Gould 1983) and since marsupial offspring are often raised in a pouch, mothers may not need to recognise their offspring until the young has permanently left the pouch. Because mothers were aggressive towards non-offspring young-at-foot that approached (Chapter 4), females must develop recognition at some point. Offspring recognition likely occurs via olfaction (Lévy and Keller 2009) and the ontogeny of this behaviour could be tested in captivity using odours.

I found that sub-adult males showed a cyclic pattern of association with adult females without young-at-foot, starting with a decrease in the breeding season, followed by an increase after about 2 months (Chapter 2). This pattern was mirrored by movements away from their mothers at around 18–25 months of age (Chapter 3). It could be that sons were avoiding aggressive adult males that were interested in their mothers in the breeding season, rather than avoiding their mothers *per se*. Alternatively, sons may move away from their mothers to associate with their peers (Bon and Campan 1996), although the lagged association rates showed that they did not choose to associate with particular individuals (Chapter 2). Small adult males did form somewhat persistent associations with other small males (Chapter 2). The developmental behaviour of young adult males could thus be investigated to determine whether alternative reproductive strategies exist compared to the dominance/tending strategy of large males (Clutton-Brock 1989).

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APPENDIX 1: A capture technique for free-ranging eastern grey kangaroos (*Macropus giganteus*) habituated to humans

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A capture technique for free-ranging eastern grey kangaroos (*Macropus giganteus*) habituated to humans

W. J. King[A,D], *M. E. Wilson*[B], *T. Allen*[B], *M. Festa-Bianchet*[C] *and G. Coulson*[B]

[A]Biology Department, Bishop's University, Sherbrooke, Québec, J1M 0C8, Canada.
Present address: School of Biological Sciences, University of Queensland, St Lucia, Qld 4072, Australia.
[B]Department of Zoology, The University of Melbourne, Vic. 3010, Australia.
[C]Département de Biologie, Université de Sherbrooke, Sherbrooke, Québec, J1K 2R1, Canada.
[D]Corresponding author. Email: wendy.king@uqconnect.edu.au

Abstract. Available methods to capture free-ranging kangaroos differ in ease of use, selectivity, risk of injury and suitability to specific environments. We describe a simple technique involving the syringe from a 'jabstick' attached to an extendable, aluminium pole. We also examine responses of eastern grey kangaroos (*Macropus giganteus*) to a range of doses of Zoletil®. We captured 307 eastern grey kangaroos that were habituated to humans in Victoria, Australia, from November 2007 to October 2009. We approached kangaroos on foot, and injected the hind limb muscle mass with the pole syringe extended up to 4.85 m. We used Zoletil® 100 at a dose rate of 4.1 ± 1.3 mg kg^{-1} (mean $\pm$ s.d., $n = 274$). Induction was rapid (4.3 ± 2.0 min, $n = 185$) and only weakly related to dose ($r^2 = 0.06$). There was no clear relationship between age, sex or body condition and induction time. This pole syringe technique can be successfully and safely used wherever animals can be approached closely, regardless of body condition. The technique provides an effective means to immobilise habituated kangaroos for research and management.

Introduction

Marked individuals are required for many studies of the ecology, behaviour and management of wild animals. Methods used to capture free-ranging macropods differ in ease of use, selectivity, risk of injury to both the animal and the handler, and suitability to specific environments (Coulson 1996). Researchers often use syringe darts, propelled by blowpipe, compressed gas, powder charge or bow string, to administer immobilising compounds into the haunch of kangaroos (Higginbottom 1989; Roberts *et al*. 2010). Disadvantages of darting, however, include injuries from high-velocity dart impact and dartguns may be unsuitable or illegal in semiurban areas. Restraining chemicals can also be administered through baiting of grain or water at habitual feeding or drinking points (Arnold *et al*. 1986) but because there is no control over the amount of drug consumed, animals show varied responses from underdosing to overdosing. An additional drawback to baiting is that non-target species may consume the bait.

Alternative methods for physically immobilising large macropods include cannon netting (Edwards *et al*. 1994), drawstring traps in fences (Coulson 1996; Coulson *et al*. 2003), 'stunning' (Robertson and Gepp 1982) and herding (Jarman and Taylor 1983). There is considerable risk of injury to the animal during immobilisation in all the above methods. Stunning also poses definite risk of injury to the catchers, who run in darkness and tackle a kangaroo, as does herding, where kangaroos are driven into nets and caught by hand.

Chemical restraint using a 1 : 1 mixture of tiletamine hydrochloride and zolazepam hydrochloride (commercially available as Zoletil® or Telazol®) has been used for over three decades (Eads 1976; Boever *et al*. 1977) on a variety of mammals. Tiletamine is a dissociative anaesthetic that provides analgesia while zolazepam is a benzodiazepine tranquiliser that acts as an anticonvulsant and muscle relaxant (Lin *et al*. 1993). This drug combination provides relatively quick induction times but long recovery periods. It has been used in free-ranging primates (Glander *et al*. 1991), carnivores, particularly ursids (Stirling *et al*. 1989), and ungulates such as cervids (Chai and le Gendre 2001), often in combination with a reversible agent, such as xylazine. A wide range of doses of tiletamine–zolazepam (2–25 mg kg^{-1} body mass: Schobert 1987) can be safely administered to marsupials, including eastern grey kangaroos (*Macropus giganteus*) (Viggers and Hearn 2005; Roberts *et al*. 2010).

In some places, kangaroos are habituated to people and will tolerate close approach. Here we describe a simple technique for use in these situations, involving the syringe from a 'jabstick', commonly used by veterinarians, attached to an extendable pole. We also assess responses of eastern grey kangaroos to various dose rates of Zoletil® and investigate the effects of sex, age, body condition and female reproductive state on induction time.

Materials and methods

We immobilised eastern grey kangaroos from November 2007 to October 2009 at two sites in Victoria: Anglesea Golf Club

10.1071/AM10029 0310-0049/11/010047

(38°24′S, 114°10′E) and Wilsons Promontory National Park (38°57′S, 146°17′E). We approached on foot, and injected kangaroos using a 10-mL Paxarms syringe (Timaru, New Zealand) attached to the end of a pole (Fig. 1). We used one of two telescopic aluminium poles; the shorter pole (1.35 m) consisted of one extension (Wildlife & Animal Capture, Warwick, Qld, Australia) and a longer pole (4.85 m) consisted of two extensions (Ettore 'REA-C-H' telescopic pole, Alameda, CA, USA). We spray-painted the two distal sections black so that the long pole would be less reflective at night.

We loaded the syringe with Zoletil® 100 (100 mg mL^{-1} of tiletamine hydrochloride–zolazepam hydrochloride mixture; Virbac Pty Ltd, Milperra, NSW, Australia) and approached kangaroos while they held their head down and their back horizontal to the ground. We pushed the syringe into the hind limb muscle mass so that the drug was injected as the needle punctured the skin. Upon injection, kangaroos usually hopped a few steps and resumed their activities or stood upright. We waited for the animal to assume lateral recumbency and approached after an additional 2–5 min, to allow the drug to take full effect. If the animal was not completely immobilised at this point (26% of captures), we administered an additional dose using a 3-mL hand-held syringe (Fig. 1), giving the same as the initial dose or a half dose, depending on the behaviour of the animal. We defined induction as the time (in minutes) elapsed between the first injection and recumbency, because the latter was easier to detect than other stages of induction, such as initial ataxia or complete inability to move. In the rare cases when the animal was recumbent, stood up and then was recumbent again, we used the second recumbency as our measure of induction.

Occasionally, we undertook captures after dark at the national park (31 captures or 10%). One of us approached the kangaroo with the pole syringe and a headlamp to distract the animal. Another person followed behind and shone a spotlight on the kangaroo once it had received an injection, to ensure that it was not lost from sight before lying down.

We fitted immobilised animals with collars and Allflex eartags (Capalaba, Qld, Australia) for individual identification. We marked large pouch young (>950 g) with small Leader eartags (Craigieburn, Vic., Australia) after their mothers had been immobilised. After taking standard body measurements (Poole *et al*. 1982), we left kangaroos to recover in a heavy jute bag in the shade and sheltered from adverse weather. Kangaroos took ~1–3 h to recover from the drug but recovery times were not monitored to avoid disturbance. There were no wild dogs in either study area although there were red foxes (*Vulpes vulpes*). Our handling procedures were approved by The University of Melbourne Animal Ethics Committee (AEC 06146 and AEC 0810628.1).

We injected Zoletil® 100 at an initial dose rate of 4.1 ± 1.3 mg kg^{-1} (mean ± s.d., $n = 274$). The drug volume ranged from 0.3 to 3.1 mL, based on our visual estimate of body mass, and was equivalent to 0.8–10.0 mg kg^{-1}. In general, subadults that appeared to weigh 10–18 kg received ~0.6–0.8 mL, adult females 1.0–1.2 mL and adult males 1.5–2.5 mL.

We estimated body condition separately for each sex as the standardised residual of the linear regression of logged body mass on hind leg length (Schulte-Hostedde *et al*. 2005). The regression equations were log(mass) = 0.00256(leg length) + 0.125 for males ($n = 60$, $r^2 = 0.95$, $P = 0.0001$) and log (mass) = 0.00252(leg length) + 0.144 for females ($n = 263$, $r^2 = 0.84$, $P = 0.0001$). We carried out statistical analyses (simple and multiple regressions using linear models and *t*-tests) with R ver. 2.9.1 (R Development Core Team 2009). The response variable was induction time while predictors included initial dose, sex, age (subadult or adult), body condition, presence of young in the pouch, and site. Males were considered to be subadults up to 25.5 kg body mass, while females were subadults up to 18.5 kg body mass. We included several two-way interactions with site, because kangaroos appeared easier to approach at the golf course than at the national park. We also performed a separate analysis for adult females to assess whether or not the presence of a pouch young affected induction time. We chose the best linear model in multiple regressions according to Akaike's Information Criterion (Burnham and Anderson 2004). When models differed by less than two units, we selected the most parsimonious model (Quinn and Keough 2002).

Results

We captured kangaroos 194 times over 49 days at the Anglesea Golf Club using mostly the shorter pole, and 113 times over 37 days at the Wilsons Promontory National Park using the long pole. In total, we captured 237 adult females, 17 subadult

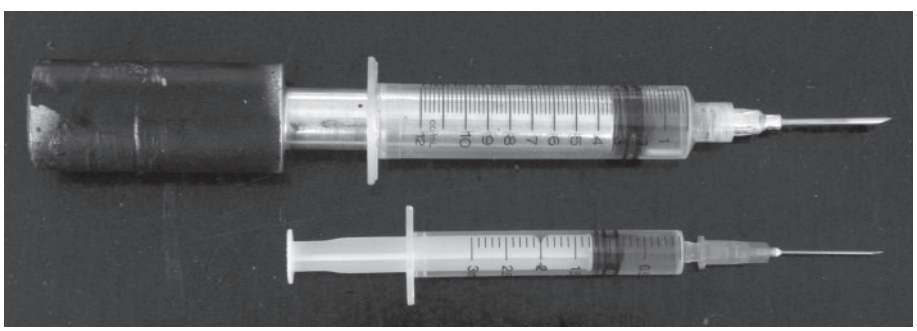

Fig. 1. The 10-mL Paxarms pole syringe (upper), consisting of a metal base (55 mm long), a metal plunger (75 mm long) with a black rubber piston and a 1.6 × 25-mm needle, compared with a standard 3-mL syringe (lower) loaded with a 0.8 × 25-mm needle.

females, 35 adult males and 18 subadult males. Most recaptures were of previously marked adult females. Since the number of injections required ($n = 43$ records), initial dose ($n = 38$) and induction time ($n = 12$) did not differ significantly between first and second captures (paired t-tests, $P \geq 0.18$), all 307 captures were considered in the analyses. Sample sizes differ among comparisons because, in some cases, incomplete first injections or inability to monitor time to recumbency did not allow us to measure all variables for the same individuals.

When the barrel of the syringe broke (23 times), we could not estimate the amount of drug administered or calculate doses. We also did not consider induction times for those captures. Of 237 adult females captured, 69% were carrying pouch young and we marked 83 large young. There were no injuries and only one death. We injected a female at the national park that appeared very emaciated, had a condition score of –0.116 (the lowest of all females captured) and did not recover from immobilisation. All of the animals captured at the golf course were seen alive three months later, and all but two in the national park were seen alive two months later. One pouch young was abandoned (mass = 1.2 kg); all others were seen in their mother's pouch after she had recovered from the drug.

Induction time averaged 4.3 ± 2.0 min ($n = 185$). In a multiple regression on induction time, the only factors retained in the best model were dose and site, with a significant interaction between dose and site ($R^2 = 0.12$) (Table 1). This was due to a negative relationship between dose and induction time at the national park, but not at the golf course (Fig. 2). Sex, age and body condition (Fig. 3) were not included in the final model. The presence of a pouch young was not included in the final model when considering only adult females ($R^2 = 0.09$) (Table 2). Again, the only factors retained in the final model for adult females were dose, site and their interaction. Kangaroos at the golf course received lower doses on average (3.9 ± 1.2, $n = 179$ versus 4.4 ± 1.3 mg kg^{-1}, $n = 95$, $t = –3.14$, $P = 0.002$) and took ~1 min longer to respond to the drug (4.8 ± 2.2, $n = 98$ versus 3.7 ± 1.7 min, $n = 87$) although there was considerable variation in individual response at both sites (Fig. 2).

Discussion

The extendable pole syringe was effective at capturing kangaroos in two sites where animals were habituated to humans. Potential sites where the technique could be used include

Table 1. Multiple regressions for several linear models of induction time for eastern grey kangaroos immobilised with a pole syringe at two sites (Anglesea Golf Club and Wilsons Promontory National Park) in Victoria (n = 182)

The equation for the selected model is INDUCTION = 0.71(DOSE) + 1.68(SITE) – 0.63(DOSE*SITE) + 2.85. AIC = Akaike's Information Criterion, ΔAIC = the difference in the value of AIC between the model and the chosen model (in bold). SITE was coded as 1 for the golf course and 2 for the park

Response variable	Predictors	% deviance	AIC	ΔAIC
Induction time (min)	DOSE+SITE+AGE+SEX+CONDITION+ DOSE*SITE+SEX*SITE+CONDITION*SITE	13.4	763.1	+1.8
	DOSE+SITE+AGE+SEX+DOSE*SITE+SEX*SITE	13.7	760.5	–0.8
	DOSE+SITE+AGE+DOSE*SITE	12.9	760.3	–1.0
	DOSE+SITE+DOSE*SITE	**11.9**	**761.3**	**0**
	DOSE	5.5	772.2	+10.9
	SITE	6.6	781.1	+19.8

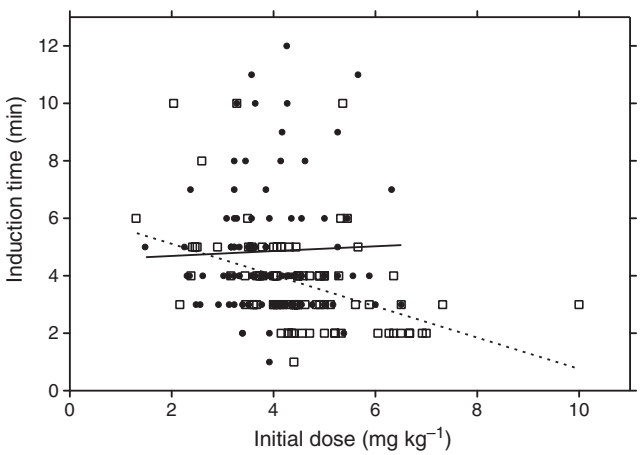

Fig. 2. Time to lateral recumbency versus initial dose of Zoletil® for 95 eastern grey kangaroos at Anglesea Golf Club (● and solid line, $r^2 = 0.001$) and 87 kangaroos at Wilsons Promontory National Park (□ and dotted line, $r^2 = 0.17$).

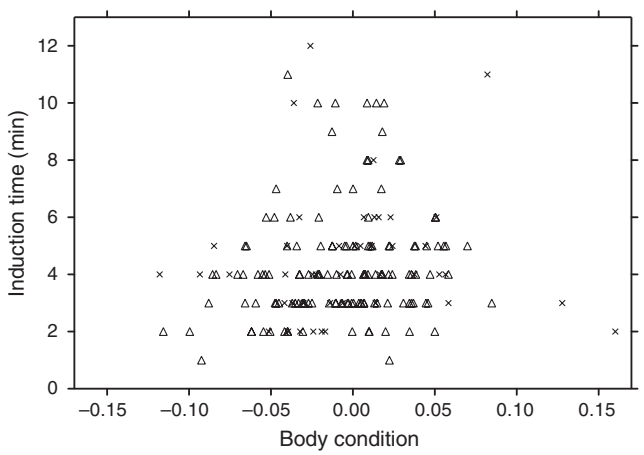

Fig. 3. Time to lateral recumbency versus body condition (standardised residuals of the linear regression of logged body mass on hind leg length) for 44 male (×) and 139 female (△) eastern grey kangaroos.

Table 2. Multiple regressions for several linear models of induction time for adult female eastern grey kangaroos immobilised with a pole syringe at two sites (Anglesea Golf Club and Wilsons Promontory National Park) in Victoria (n = 125)

The equation for the selected model is: INDUCTION = 0.84(DOSE) + 2.50(SITE) – 0.78(DOSE*SITE) + 2.03. PY = presence of pouch young, AIC = Akaike's Information Criterion, ΔAIC = the difference in the value of AIC between the model and the chosen model (in bold). SITE was coded as 1 for the golf course and 2 for the park

Response variable	Predictors	% deviance	AIC	ΔAIC
Induction time (min)	DOSE+SITE+PY+CONDITION+DOSE*SITE+ PY*SITE+CONDITION*SITE	6.7	529.8	+7.4
	DOSE+SITE+PY+DOSE*SITE+PY*SITE	8.0	526.2	+3.8
	DOSE+SITE+DOSE*SITE	**9.4**	**522.4**	**0**
	DOSE	5.5	531.1	+8.7
	SITE	2.7	533.9	+11.5

golf courses, fauna parks and periurban reserves, campgrounds and other places with frequent human visitation. In these areas, there is often a need to capture kangaroos for management, public safety or amenity, or for animal welfare reasons. As long as the kangaroos can be approached within 5 m, this technique is simpler than any of those previously described, which require weapons permits, safety awareness training and practice shots (darting and stunning), extensive fencing (draw-string traps), setting of nets (draw-string traps and cannon netting), careful distribution and monitoring of bait (baiting) or a coordinated team of skilled assistants (herding, stunning and cannon netting). Mortality rates have been reported as 2% for stunning (Robertson and Gepp 1982) and 4.5% for baiting (Arnold *et al*. 1986). In contrast, the pole syringe caused no injuries and only 0.3% capture-induced mortality. We suggest that kangaroos appearing in extremely poor condition should not be immobilised with this technique, except for euthanasia.

Zoletil® immobilised 82% of kangaroos within 5 min, confirming the rapid induction reported in other macropodid species. We could explain only a small proportion of the variance in induction time by considering dose and site. The relationship between dose and induction time was weak at the national park and non-existent on the golf course, where induction took ~1 min longer, on average, possibly because of the slightly larger size of kangaroos on the golf course, where we also used a slightly lower dose. Therefore, variability in individual response due to unmeasured variables appears much greater than that predicted by dosage, and increasing dosage will not necessarily lead to reliably faster induction. Mean induction time was 1.9 min in red-necked wallabies (*Macropus rufogriseus*) (von Degerfield 2005) and 7.9 min in agile wallabies (*Macropus agilis*) and 7.6 min in red kangaroos (*Macropus rufus*), where it was extremely variable among individuals (Boever *et al*. 1977; Stirrat 1997), similar to our findings.

Roberts *et al*. (2010) reported that in eastern grey kangaroos, induction time was 8.3 min with a mean dose of 4.8 mg kg^{-1}, with no effect of body mass on induction. We found that dose affected induction time at only one study area in a multivariate analysis. Body mass and condition do not appear to have a strong effect on induction time for Zoletil® in kangaroos.

We did not measure duration time because recovery included a phase when the animal was able to stand but was very wobbly and prone to falls. In both study sites, the presence of an observer would have stimulated behaviours that could have led to injury to the animal or to a pouch young when present. On the golf course, we placed animals in sites as hidden from view as possible to reduce the chance of encounters with golfers during recovery.

Optimal dose rates of Zoletil® vary among species (Lin *et al*. 1993), being 4.1 mg kg^{-1} for red kangaroos (Boever *et al*. 1977), ~10 mg kg^{-1} for agile wallabies (Stirrat 1997) and 12.9 mg kg^{-1} for red-necked wallabies (von Degerfield 2005), consistent with decreasing dose rates as mean adult size increases (Schobert 1987). A dose of 5 mg kg^{-1} was considered an underdose for agile wallabies but 13.4 mg kg^{-1} was deemed safe (Stirrat 1997). We used a mean dose rate of 4.1 mg kg^{-1}, which provided immobilisation for the approximate 30 min required to attach a collar and eartags and take body measurements. Increasing the dose would increase the cost and possibly increase the risk to the animals but would probably have little effect on induction time.

We found no differences in the response of males and females. Sexual differences reported in the literature are inconsistent. Female agile wallabies had shorter induction times than males, but males appeared more deeply sedated than females (Stirrat 1997).

In conclusion, this technique is very safe and effective where kangaroos can be approached to within 5 m, regardless of body condition. It provides an effective means to immobilise habituated kangaroos for research and management.

Acknowledgements

We thank all volunteers who assisted with kangaroo captures. Funding was received from the Department of Sustainability and Environment, the Holsworth Wildlife Research Endowment, the Senate Research Committee of Bishop's University, the Natural Sciences and Engineering Research Council of Canada and the Australian Research Council.

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Manuscript received 1 September 2010, accepted 18 January 2011